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Trichothecium roseum Enters 'Fuji' Apple Cores Through Stylar Fissures

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Abstract

Apple core rot, an economically important disease worldwide, appears both before and during harvest. Current gaps in understanding of the infection cycle impede progress toward more effective management of this disease. The fungus *Trichothecium roseum* is the main pathogen of core rot on apple in China. In this study, we used fluorescent labeling to trace colonization of *T. roseum* in floral tissues, characterizing routes of penetration to the core of 'Fuji' apples. *T. roseum* infected petals, anthers, filaments, stigmas and separated styles of flowers, and floral debris served as inoculum for core infection. In field inoculations, *T. roseum* entered styles initially through stylar fissures and colonized pluricellular hairs of these fissures during early stages of fruit development. Subsequently, hyphae grew along the extending fissures, which are continuations of stylar fissures located between stylar bases and carpel cavities. The hyphae remained in the extending fissures from mid-June to late July. When fruit developed an open sinus in late July, the sinus eventually fused with extending fissures and carpel cavities in late August, hyphae invaded carpel cavities, and ultimately fruit flesh via cracks on carpel cavity walls. Our results revealed for the first time the routes by which *T. roseum* penetrates apple fruit, and provided significant insights for strategic management of core rot.

Keywords: apple, core rot, moldy core, *Trichothecium roseum*, stylar infection, infection route, fungi, fruit, tree fruits, epidemiology, disease development and spread

Core rot and moldy core of apple are economically important fruit diseases that cause preharvest fruit drop, core rot and moldy core on mature fruit, and fruit decay in storage (Serdani et al. 1998; Spotts 1990). In the apple production area of southeastern Gansu Province, China, incidence of diseased fruit (cv. Delicious) was commonly 40 to 50% at harvest, and as high as 70% in the most severely affected orchards (Hu et al. 1996). In northern Israel, incidence of core rot in 'Red Delicious' averaged 3 to 10%, with a peak of 40% in some orchards (Niem et al. 2007; Reuveni 2006; Reuveni et al. 2002). In Australia, 5% of stored 'Fuji' apple fruit exhibited moldy core (Kupferman 1992).

Efficacy of control of core rot and moldy core with fungicides applied in bloom and during fruit maturation has been inconsistent. In Tasmania, incidence of core rot was <1% when two benomyl and iprodione sprays were applied during the full-bloom stage (Archer 2002). In Israel, applying difenoconazole or bromuconazole with captan at the pink-cluster, 60% bloom, and full bloom stages reduced incidence of moldy core by 40 to 60% compared with untreated trees (Reuveni and Prusky 2007). However, failures of fungicide applications have been reported as well. In Ohio of U.S.A., 13 fungicide applications from bloom to about 1 month before harvest failed to provide satisfactory control of moldy core (Ellis and Barrat 1983). In another Israeli study, incidence of moldy core and core rot was not suppressed by application of bromuconazole and captan in bloom (Shtienberg 2012).

Moldy core and core rot are disease complexes, and causal fungi vary among geographic regions. In China, the most common fungi associated with moldy core are *Alternaria* and *Cladosporium* spp., and core rot is mainly caused by species of *Trichothecium*, *Epicoccum*, *Phoma*, *Fusarium*, and *Penicillium* (Gao et al. 2013). In Oregon, U.S.A., fungal genera associated with moldy core included *Alternaria*, *Stemphylium*, *Cladosporium*, *Ulocladium*, *Epicoccum*, *Coniothyrium*, and *Pleospora* (Spotts 1990). Fungi that were associated with core rot of 'Starking' apple fruit in South Africa included *Alternaria alternata*, *Pleospora herbarum*, *Coniothyrium* sp., *Penicillium funiculosum*, *P. expansum*, and *P. ramulosum* (Combrink et al. 1985; van der Walt et al. 2010).

Symptoms of infected fruit include moldy core, core rot, and browning (Gao et al. 2013). Core rot is further partitioned as wet core rot and dry core rot, depending on the presence or absence, respectively, of rot in fleshy tissues surrounding the core. Dry core rot was associated with *Coniothyrium*, *Epicoccum*, and *Phoma*, whereas wet core rot was associated with *Trichothecium*, *Fusarium*, and *Penicillium* (Combrink et al. 1985; Gao et al. 2013; van der Walt et al. 2010). Mixed infections are common in this disease complex, and symptom appearance can also vary with inoculum dose (Gao et al. 2013).

Core rot and moldy core occurred mainly in so-called open core cultivars, in which the apple core has an open connection to the calyx tube (Bell 1940; Harshman and Evans 2015; Miller 1959). The apple sinus is a passageway between the core cavities and calyx tube in open core cultivars (Spotts et al. 1988, 1999). Several factors, including weather conditions (Miller 1959), crop load (Carne et al. 1929), fruit shape (Spotts et al. 1988), and heredity (Spotts et al. 1999), influence the degree of fruit sinus opening. In open core cultivars, disease incidence was positively correlated with the extent of sinus opening (Hu et al. 1995b). Hu et al. (1995b) found that 'Starking', 'Delicious', 'Starkrimson', and 'Well Spur' apples, which had a relatively open sinus, experienced from 44.0 to 56.5% disease incidence, whereas 'Fuji' apples, with narrower sinus openings, had 12.5% disease incidence. Fruit with an open calyx tube also increased the likelihood of moldy core incidence (Harshman and Evans 2015). These authors showed that, in open core cultivars, disease incidence

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of fruit with a closed calyx was 10.8%, whereas disease incidence of fruit with an open calyx was 16.7%.

Previous studies focused mainly on core infection by *Alternaria* spp. in 'Red Delicious'. The infection route to the apple core was inferred by the colonization rate of flower tissues and core regions at various stages of disease development. Ellis and Barrat (1983) presumed that the pathogen entered the receptacle or core region of fruit through the open calyx tube, based on their finding that *Alternaria* sp. colonized flower parts during or shortly after bloom and later moved into the fruit core. Based on an observed increase in the incidence of colonization of carpel cavities beginning 3 weeks after petal fall, Hu et al. (1995a) suggested that *A. alternata* initially colonized styles on apple blossoms and then entered the core region through the opening or loose tissue between the end of the calyx tube and the carpel cavity. Nevertheless, objective evidence clarifying infection routes of pathogens into the apple core has not been produced.

In addition to the sinus, some fissures form during apple fruit development. In apple flowers, the style belongs to the 'half unity' type: an upper portion with five independent styles, which is termed a 'separated style', and a lower portion consisting of a single connate style that is referred to as a 'unified style' (Cresti et al. 1980). In the inside of a unified style, there are several stylar fissures filled with pluricellular hairs (Bell 1940). Whether stylar fissures are an infection route for moldy core and core rot pathogens remains unclear.

China is the top producer of apples in the world. 'Fuji' is the predominant cultivar, accounting for approximately 70% of apple production (Li et al. 2014). Apple core rot occurs in all main production zones in China, and *Trichothecium roseum* (Pers.) Link is the main pathogen of core rot (Gao et al. 2013) (Fig. 1). The objective of this study was to determine the pathways by which *T. roseum* hyphae enter and infect the core of apple fruit, in order to provide new insights leading to more effective disease management.

Materials and Methods

Inoculum preparation. Isolate TR45 of *Trichothecium roseum*, obtained from a diseased apple fruit in Yangling, Shaanxi Province, China, was identified by morphological and molecular phylogenetic analysis (Fig. 1; Supplementary Fig. S1). The sequence of large subunit (LSU) (Accession number: MN577282) was deposited in GenBank. Its pathogenicity was verified by fulfilling Koch's postulates. Isolate TR45 was used to produce the GFP-tagged *T. roseum* used in this study. GFP expression was achieved by protoplast transformation with the GFP expression vector pRM7 where GFP was driven by the constitutive promoter RP27 (Dai et al.

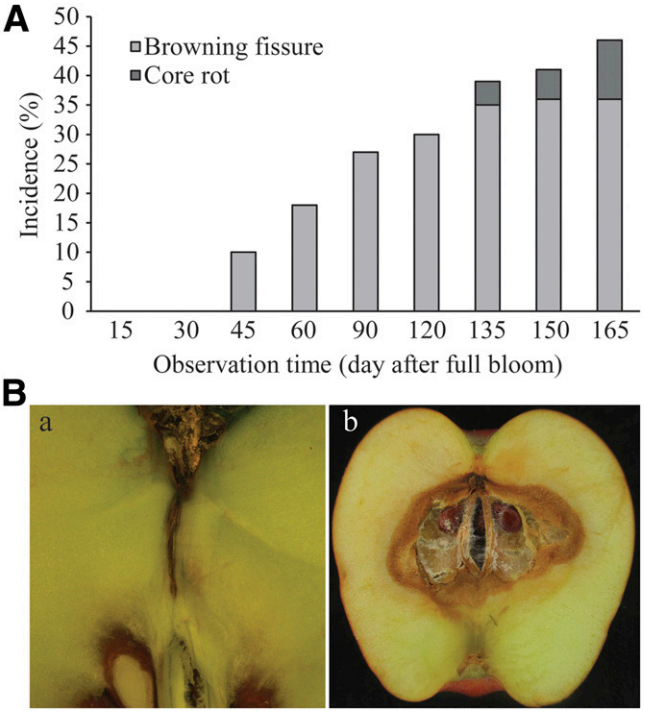

Fig. 2. Disease development in the interior of developing apple fruit (cv. Fuji) from 15 to 165 days after full bloom (dafb) when conidia of *Trichothecium roseum* were inoculated during bloom. **A,** Incidence of browning fissures and core rot during the course of fruit development. At least 60 fruit were used to calculate incidence at each fruit stage in 2 years of monitoring. **B,** Symptoms of browning fissures (a) and core rot (b).

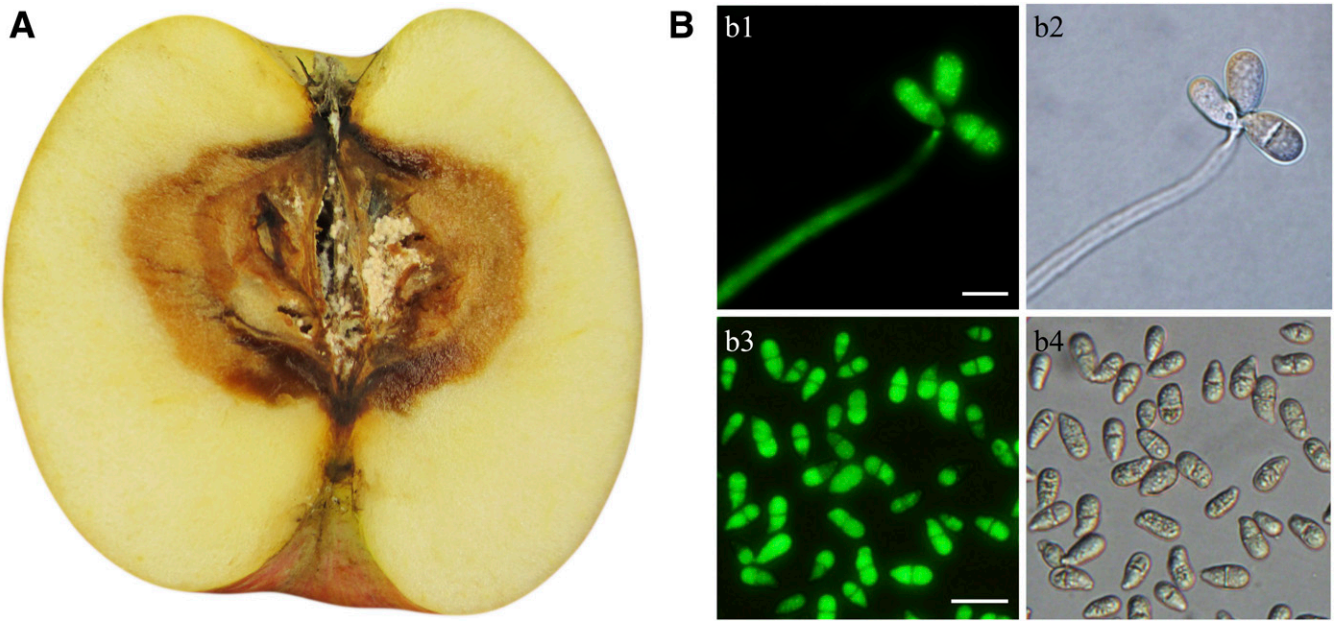

Fig. 1. Morphology of *Trichothecium roseum* and symptoms of Trichothecium core rot on apple. **A,** Fruit flesh adjacent to the apple core exhibited a brown wet rot. **B,** Conidiophores and conidia of isolate TR45 labeled with GFP. b1, b3 were under fluorescent microscopy. b2, b4 were under differential interference contrast (DIC) microscopy. Bar b1, 2 = 10 μm; b3, 4 = 20 μm.

2019a). This GFP isolate was maintained on potato dextrose agar (PDA).

To harvest conidia, colonies of the labeled isolate grown on PDA for 6 days at 25°C in darkness were flooded with 10 ml sterile water, scraped, and filtered through one layer of Miracloth (Calbiochem, U.S.A.). For inoculation, the conidial suspension was adjusted to a concentration of 5×10^6 ml^{-1} using a hemocytometer and amended with Tween-80 at a final concentration of 0.1%.

Detached flower inoculation. To study *T. roseum* flower infection, floral clusters of cv. Fuji containing symptomless, fully opened flowers were collected from orchards near Yangling. For petal inoculation, 21 petals were gently excised from flowers and about 50 μl of conidial suspension (5×10^6 conidia ml^{-1}) of the GFP-labeled TR45 was smeared on the upper surface of each petal with a soft-haired brush. An additional three petals were mock inoculated with sterile distilled water. Stamens and styles were

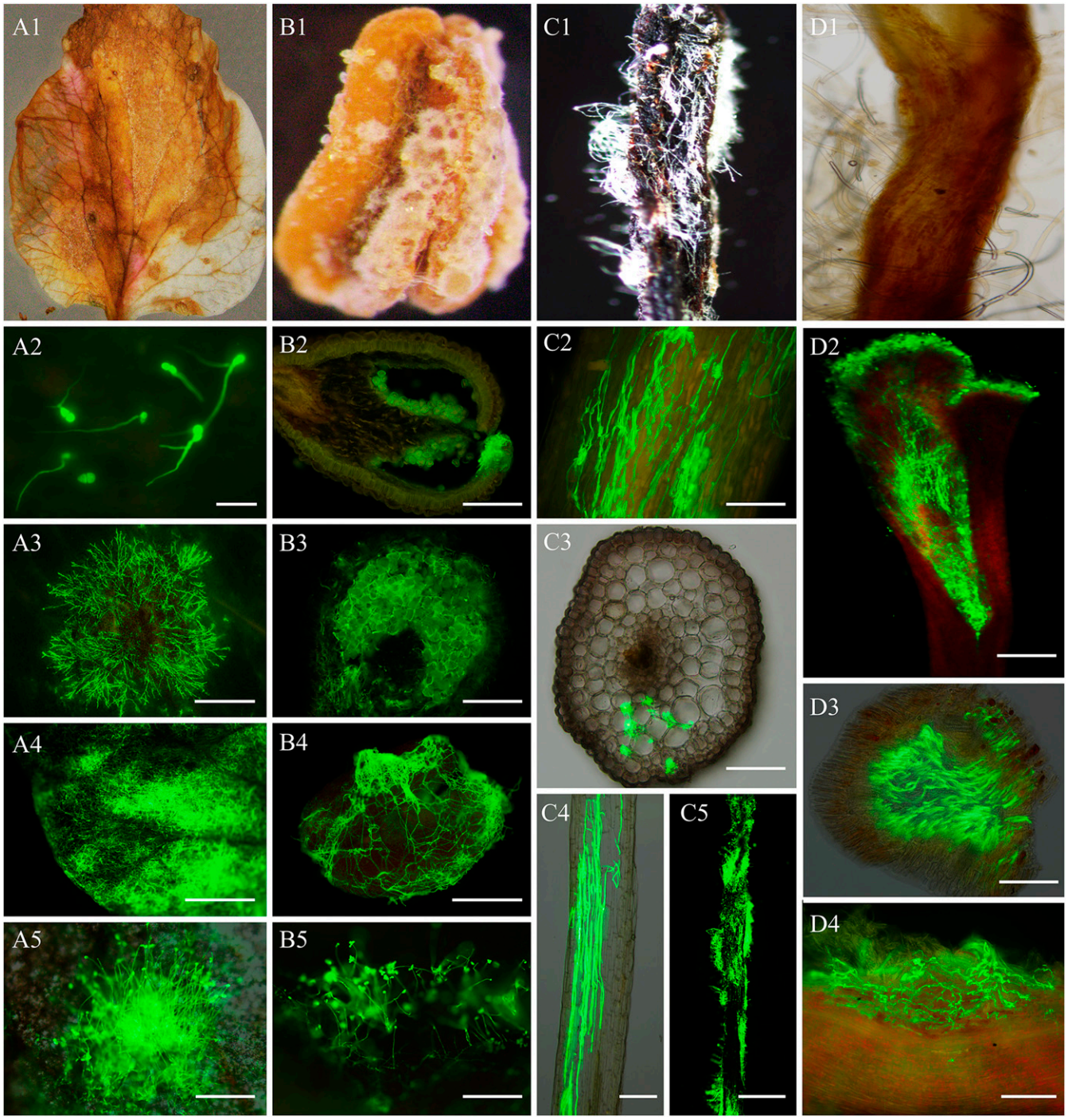

Fig. 3. Flower tissues infected by *Trichothecium roseum* isolate TR45 labeled with green fluorescent protein (GFP) following detached-tissue inoculation. **A1–A5,** Infection of petals. A1, Petal blight. A2, Germinated conidia on the surface of petal at 16 h postinoculation (hpi). A3, Hyphae ramified on the petal surface at 48 hpi. A4, Petal tissues entirely colonized at 60 hpi. A5, Sporulation on the petal surface at 96 hpi. **B1–B5,** Infection of anthers. B1, Mummified anther. B2, Hyphae extending into anthers via stomium of anther at 30 hpi. B3, Pollen grains enclosed by hyphae in the anther at 60 hpi. B4, Anthers enclosed by hyphae at 84 hpi. B5, Sporulation on the anther at 96 hpi. **C1–C5,** Infection of filaments. C1, Diseased filament. C2, Hyphae colonizing filament surface. C3, Hyphae invading the filament interior (transverse section). C4, Hyphae inside the filament (longitudinal section). C5, Hyphae extending to the surface of the filament at 96 hpi. **D1–D4,** infection on the styles. D1, Diseased style. D2, Hyphae in the inside of the stigma. D3, Hyphae inside the style (transverse section). D4, Hyphae ramifying on the style surface. A5, C3, C4, D3 were merged from multiple images made by differential interference contrast (DIC) and epifluorescence. Bars: A2 = 20 μm; C2, D3 = 50 μm; B2, B3, C3, C4, D4 = 100 μm; A3, A5, B4, B5, D2 = 200 μm; A4, B4, C5 = 500 μm.

inoculated on flowers from which the petals had been removed; a 50-μl droplet of conidial suspension (5×10^6 conidia ml^{-1}) was placed on each stamen using a pipette, and 50-μl droplets were also placed on each style. A total of 21 flowers were inoculated and an additional three flowers were mock inoculated with sterile distilled water. The inoculated and mock-inoculated petals and flowers were placed in 8.5-cm-diameter petri dishes (each dish including three petals and flowers) with sterilized filter paper that had been moistened with sterile distilled water and incubated at 25°C in darkness. Inoculated petals, stamens, and styles were taken from the incubator and observed for infection by fluorescent microscopy (Olympus BX51, Japan; X-Cite 120Q) at 6, 12, 18, 24, 48, 72, and 96 h postinoculation. Three inoculated petals and flowers were observed per time point. Each experiment was performed three times.

Field inoculation. Field experiments were conducted in an apple orchard in Yangling in 2015 and 2016. The experimental trees were cv. Fuji on apple dwarf rootstock M.9 and were 7 years old in 2015. Orchard management followed prevailing commercial-grower practices for irrigation and fertilization, but no fungicides were applied.

To study *T. roseum* infection route and timing of Trichothecium core rot (TCR) occurrence, *T. roseum* conidia were inoculated in bloom stage. Two fully opened flowers on a cluster were retained for inoculations, and other flowers were excised. A conidial suspension (5×10^6 conidia ml^{-1}) was sprayed on stamens and separated styles of each flower using a 20-ml spray bottle until flowers were entirely wet. About 800 flowers on 20 trees were inoculated in each year. About 200 flowers on 5 other trees were mock inoculated with sterile distilled water. Inoculated flowers were immediately wrapped

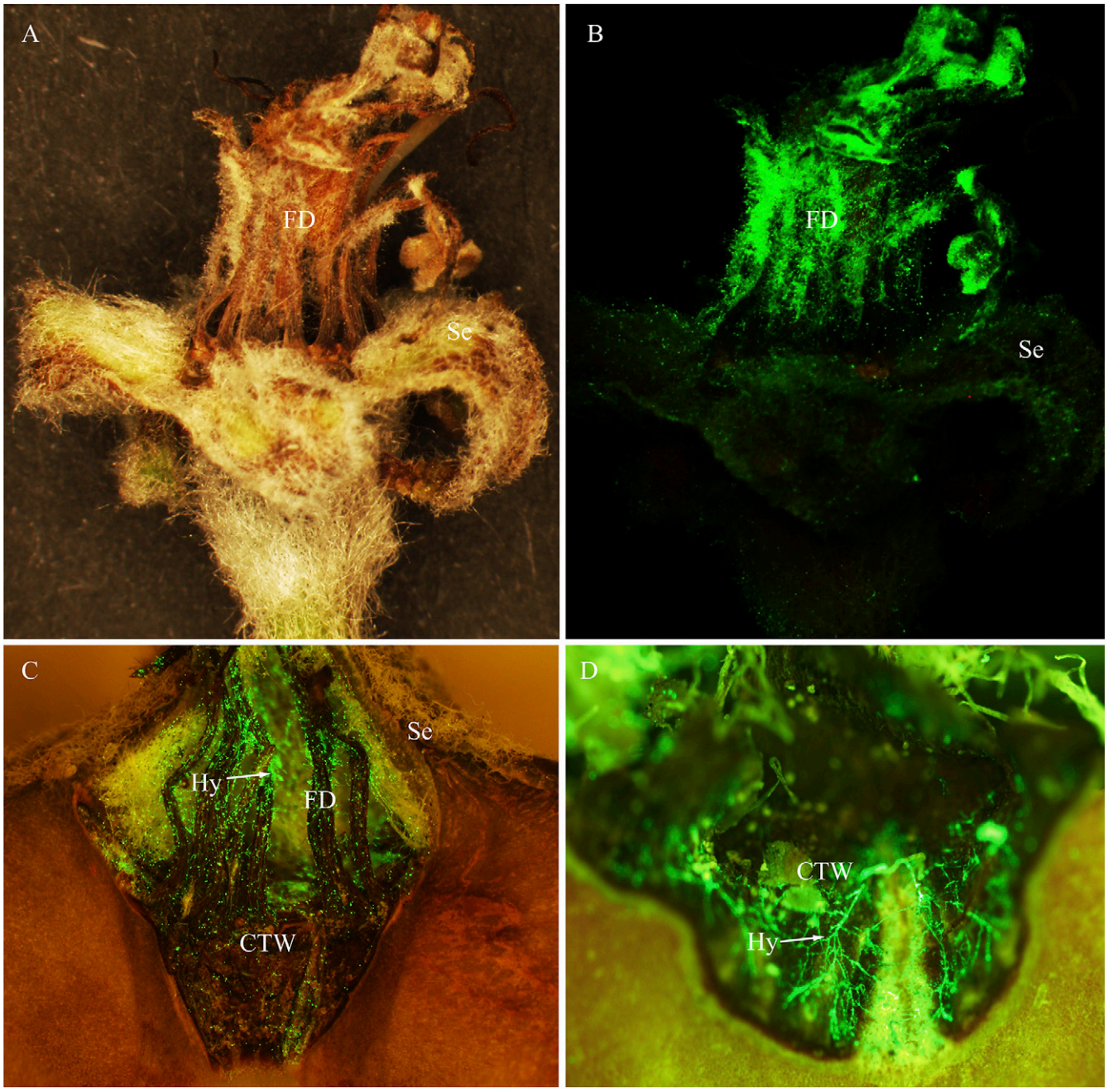

Fig. 4. Flower debris inside and outside the calyx tube infected by *Trichothecium roseum* isolate TR45 labeled with green fluorescent protein (GFP) in field inoculations. **A,** Diseased flower debris (FD). **B,** Infected flower debris on the exterior of the tissue. **C,** Hyphae (Hy) colonizing flower debris in the calyx tube, and restricted in the calyx tube by the wall. **D,** Hyphae colonizing the wall of the calyx tube but not penetrating the calyx wall. Se, sepal; CTW, calyx tube wall.

in transparent plastic bags whose inner surfaces had been sprayed with sterile distilled water to maintain high humidity, and these bags were removed after 60 h. To mark inoculated flowers, labels with inoculation time indicated were tied to the branch subtending the inoculated flowers. Inoculated flowers and fruit were collected from the orchard and observed for hyphal colonization by fluorescent microscopy (Olympus BX51, Japan; X-Cite 120Q) and fluorescent stereo microscopy (Olympus SZ61; X-Cite 120Q) at 5, 10, 15, 30, 45,

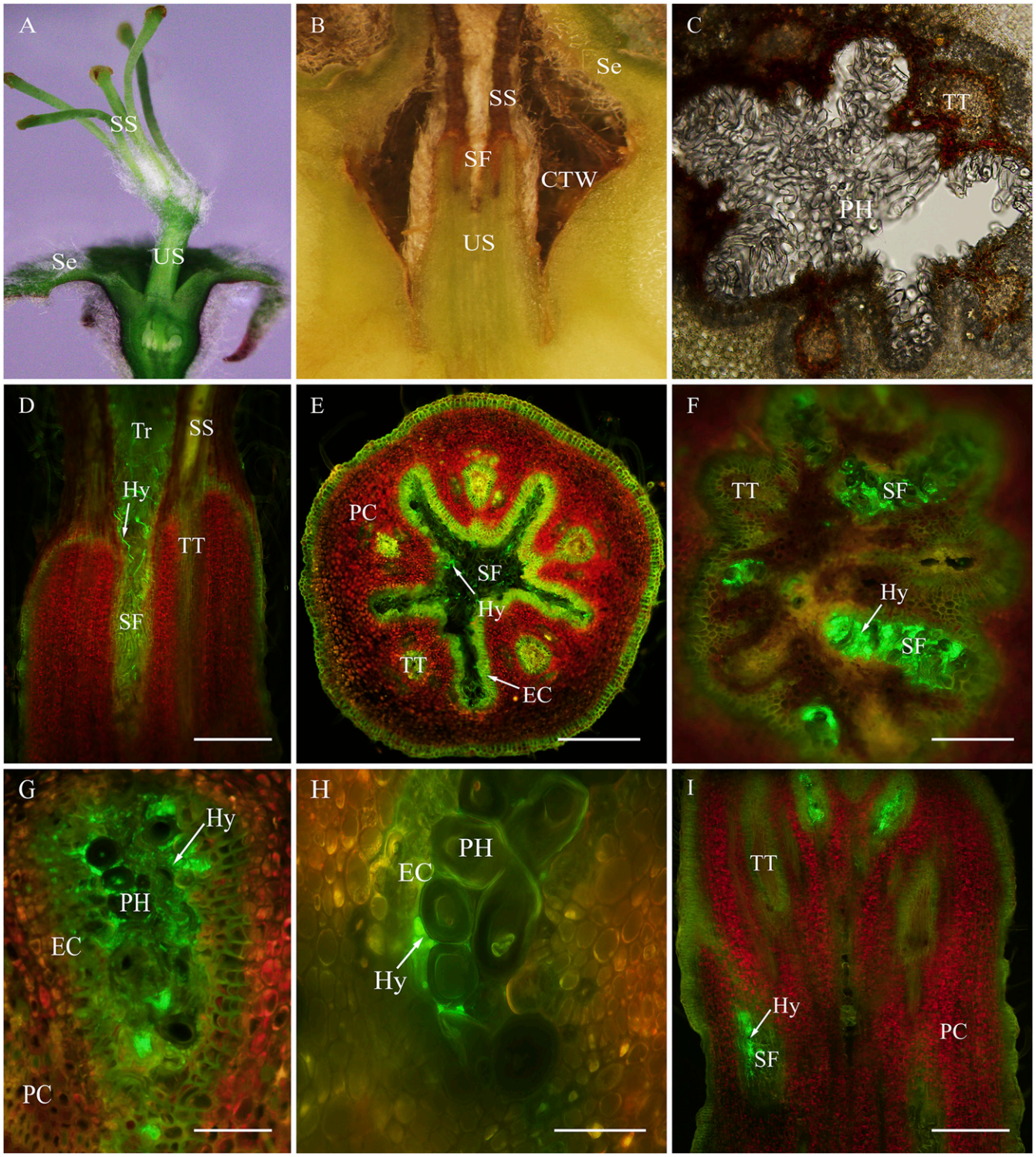

Fig. 5. Stylar fissures in the calyx tube infected by *Trichothecium roseum* isolate TR45 labeled with green fluorescent protein (GFP) in field inoculations. **A,** Five separated styles (SS) were fused at bases to form a unified style (US). **B,** A unified style when sepals were closed. Spaces between the five parts of the unified style tapered gradually to a stylar fissure (SF) that included abundant pluricellular hairs that functioned as trichomes. **C,** The top orifice of the unified style was filled with pluricellular hairs (PH). **D,** Hyphal (Hy) colonization among the trichomes (Tr), extending into the stylar fissure. **E,** Hyphae massed at integrated stylar fissures in upper parts of a unified style. **F,** Hyphae spread in separated stylar fissures to lower parts of unified styles but were limited by lignified endoepidermal cells (transverse section). **G,** Stylar fissure was filled with pluricellular hairs, and colonized by the hyphae, but hyphae did not break through lignified endoepidermal cells. **H,** Hyphae extending among pluricellular hairs but not penetrating them. **I,** Hyphae spread vertically in stylar fissures (longitudinal section) but did not invade parenchymal cells (PC) or transmitting tissues (TT). CTW: calyx tube wall, Se: sepal. Bar: D, E, F, I = 200 μm; G = 100 μm; H = 50 μm.

60, 75, 90, 105, 120, 135, 150, and 165 days postinoculation (dpi). Thirty inoculated flowers were examined at each time point. To observe stylar colonization, the unified style parts and 2-mm-long segments of adjacent tissues below style bases were separated from the inoculated apples. The unified style parts were sliced transversely and longitudinally using double-edged razor blades. For observation of ovary colonization, inoculated fruit were bisected longitudinally using a flame-sterilized knife to expose intervening tissues and fruit carpel cavities. To clarify the timing of TCR symptom development, apple fruit collected at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, and 165 dpi were bisected for observation; 30 inoculated fruit were investigated at each time point. Fruit in which *T. roseum* had visibly colonized the mesodermal cells were considered to exhibit TCR symptoms. The experiment was conducted in 2015 and repeated in 2016.

Results

Core rot symptom occurrence. For inoculated fruit that were collected from 135 days after full bloom (dafb) to 165 dafb (from early September to early October in Yangling), 4 to 10% of inoculated fruit exhibited core rot in the carpel cavities (Fig. 2A and B). In contrast, no fruit collected at 15 to 120 dafb exhibited symptoms in the carpel cavity (Fig. 2A). For fruit collected from 45 to 120 dafb, 10 to 30% of fruit exhibited 'browning fissure' symptoms in tissues between the style bases and the top of carpel cavities (Fig. 2A and B).

Infection of apple flower tissues. After detached flowers were inoculated with *T. roseum*, conidia germinated on all flower tissues except sepals (Fig. 3A2) and hyphae colonized petals, anthers, filaments, and separated styles. After infection, *T. roseum* blighted petals (Fig. 3A1), mummified anthers (Fig. 3B1), and blackened and withered filaments and styles (Fig. 4C1 and D1). Hyphae ramified over the petal surface until petal tissues were entirely colonized, followed by sporulation (Fig. 3A3–A5). Hyphae entered anthers via their stomia, then propagated and enclosed pollen grains. On the exterior surfaces of anthers, hyphae enclosed the anthers and sporulation ensued (Fig. 3B2–B5). Hyphae spread over the surface and interior of filaments, ultimately colonizing the surfaces of dead filaments as well (Fig. 3C2–C5). Hyphae entered the style from the stigma, growing both inside and on the surface of the style (Fig. 3D2–D4).

Colonization of flower debris and calyx tube of newly developing fruit. When *T. roseum* was inoculated on fully opened flowers in the field, hyphae were observed on diseased flower debris at 5 to 10 dpi (Fig. 4A and B). Parts of the diseased flower debris were wrapped in the calyx tubes of the newly formed fruit as the sepals closed (Fig. 4C). Hyphae grew on the surface of calyx walls, sometimes turning the wall black (Fig. 4D). Fruit ovaries were not infected at this stage (data not shown), and hyphae did not penetrate the calyx wall to invade fruit flesh (Fig. 4D).

Ingress and extension in styles. 'Fuji' fruit contain five separated styles in the upper part and one unified style in the lower part, and the unified style inserts into the basin of the calyx tube (Fig. 5A; Fig. 6). The narrow open spaces among separated styles tapered gradually as the stylar fissure became unified (Fig. 5B). Transverse sections of the top of unified styles showed that there was a natural orifice containing pluricellular hairs inside the unified style (Fig. 5C). We found that hyphae among the interspaces of the separated styles entered the orifice of the fissure of the unified style (Fig. 5D). Hyphae colonized mainly inside integrated stylar fissures and did not penetrate endoepidermal cell walls into parenchymal cells of styles (Fig. 5E). In lower parts of unified styles, where integrated stylar fissures branched into separated stylar fissures, hyphae extended into these separated fissures (Fig. 5F). Hyphae colonized the space surrounding pluricellular hairs in separated stylar fissures but did not penetrate into the hairs (Fig. 5G and H). Hyphae spread in fissures toward the style base (Fig. 5I).

Extension in intervening tissues. During fruit development, sinuses of 'Fuji' fruit exhibited a variation from closed to open. During early fruit growth stages (before about 70 dafb), the sinus was closed. Some extending fissures, which are continuations of stylar fissures in intervening tissues, ramified downward among carpel cavities. The extending fissures did not link with carpel cavities (Fig. 6; Fig. 7A, B, and D). At this stage, hyphae spread in the extending fissures, creating a brown discoloration of these tissues (Fig. 7A–C). Hyphae did not enter the carpel cavities (Fig. 7E). By late fruit growth stages (after about 100 dafb), the sinus of 'Fuji' fruit had gradually opened, including an open sinus in the intervening tissues; the extending fissures were torn and fused with the sinus, and the sinus extended into carpel cavities (Fig. 7F–H). Hyphae spread in the sinus toward the carpel cavities (Fig. 7G). However, some fruit never developed extending fissures or open sinuses in the intervening tissues; in these fruit, no hyphae of *T. roseum* were found in the core or fruit flesh.

Penetration of carpels. At fruit ripening stage (135 to 165 dafb), the sinus and carpel cavities of >50% of the fruit had fused together, producing an open core (Fig. 6; Fig. 8A and B). The open core provided hyphae with ready access to carpel cavities, and inoculated fruit exhibited 'browning crack' symptoms in the carpel cavities (Fig. 8C). Hyphae ramified on carpel cavity walls (Fig. 8D), massed in cracks in carpel cavities (Fig. 8E), and infected mesodermal cells beneath carpel cavities via cracks (Fig. 8F), leading to infection followed by core rot.

Limitation of hyphal expansion by host barriers. In stylar fissures, endoepidermal cells and lignified cells on the surface of these fissures separated hyphae from stylar parenchyma and limited expansion of hyphae downward into the style base (Fig. 5E–I). In extending fissures and the open sinus in intervening tissues, superficial lignified endoepidermal cells separated hyphae from fruit flesh and let hyphae grow downward into the core (Fig. 7C, E, G). Although carpel cavity walls blocked hyphae from infecting the fruit mesocarp, cracks beneath these walls caused by fruit enlargement provided pathways for invasion of the mesocarp (Fig. 8F).

Discussion

Our study revealed that hyphae of *T. roseum* invaded apple cores by: 1) colonizing flower tissues during or after bloom; 2) penetrating through fissures in the unified style and the tissues between the style base and carpel cavities; 3) entering carpel cavities via the opening sinus; and 4) infecting fruit flesh through cavity cracks, leading to core rot.

Several studies indicated that moldy core and core rot pathogens infected apple flowers (Ellis and Barrat 1983; Hu et al. 1995a; Niem

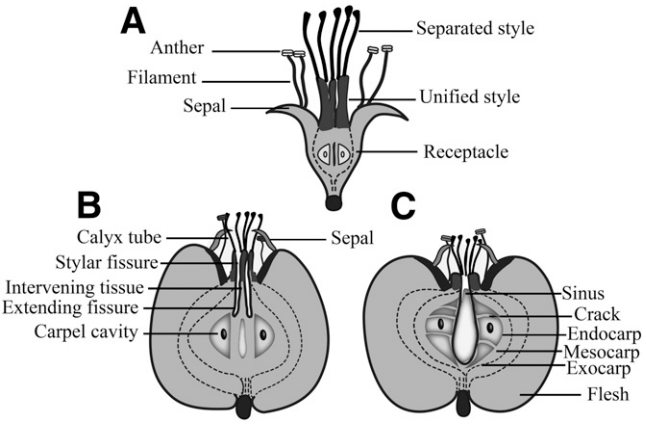

Fig. 6. Schematic diagram of structure of developing 'Fuji' apple fruit. **A,** New developed young fruit. **B,** Expanding fruit. The extending fissure in intervening tissues, a continuation of the stylar fissure. Intervening tissue is located between the stylar base and carpel cavities. **C,** Fruit with open sinus.

et al. 2007). Thus, the bloom stage was inferred to be the most important stage for pathogen infection leading to disease development. Based on our results on 'Fuji' fruit, hyphae of *T. roseum* can remain in stylar fissures and extending fissures for a considerable length of time until the sinus forms. In some fruit, in which no open sinus forms, hyphae remain restricted to these tissues and symptoms never develop. We therefore suggest that infections in bloom, even if they reach intervening tissues, may or may not lead to symptom development. The long-distance infection routes and sinus opening time slowed core rot occurrence time. Our results may help to explain

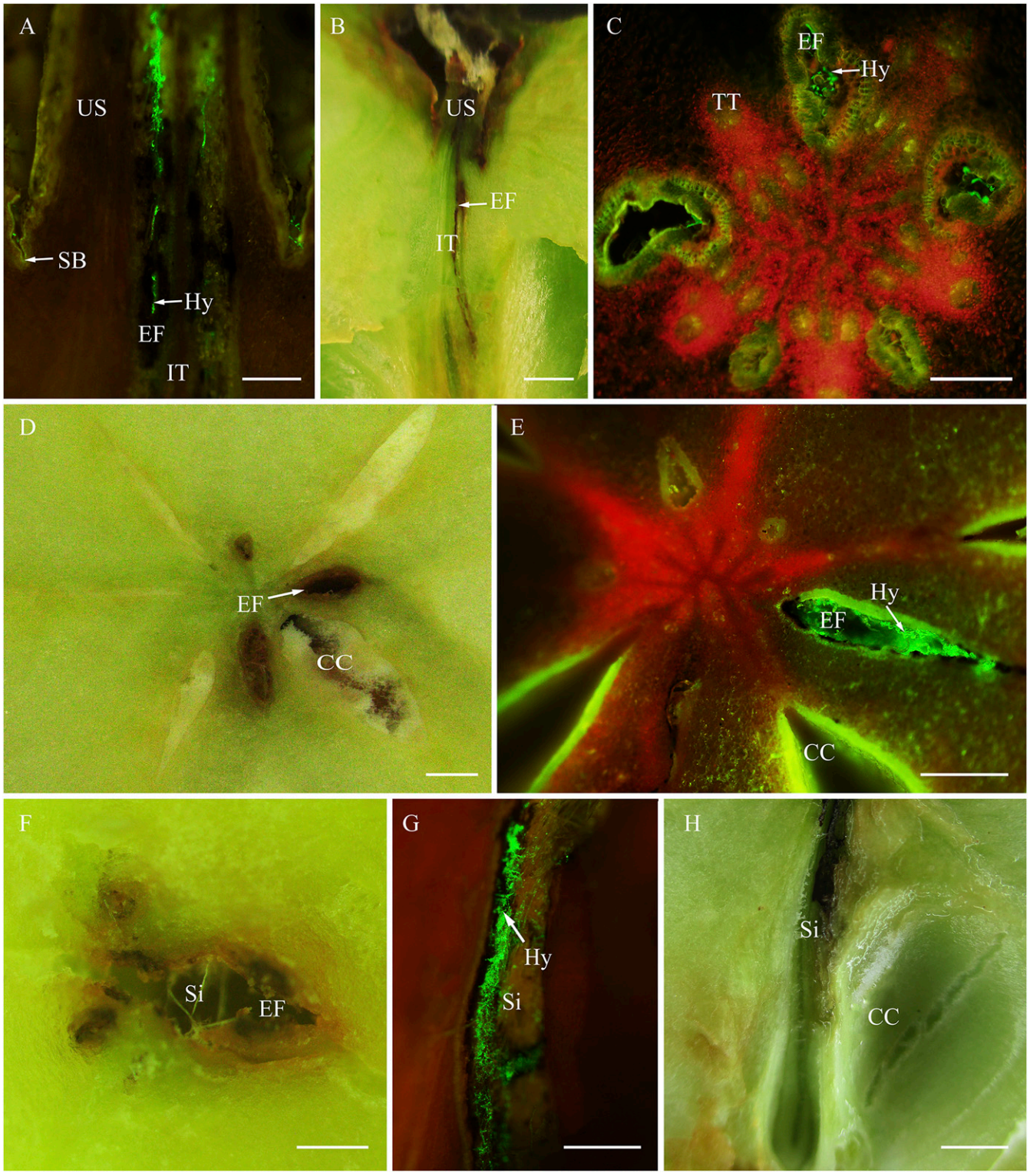

Fig. 7. Hyphal extension in the fissures and open sinus of intervening tissues. **A,** Hyphae passed the style base (SB) and entered the extending fissures (EF) in intervening tissues (IT). **B,** Extending fissure (EF) infected by hyphae, resulting in brown discoloration between unified style (US) base and the top of carpel cavity. **C,** Hyphae (Hy) colonizing the extending fissure (transverse section). **D,** Extending fissures among carpel cavities (CC) were infected and browned, and did not fuse with carpel cavities. **E,** Hyphae in the extending fissure did not enter carpel cavities (CC) before about 100 days after full bloom. **F,** Sinus (Si) opened between the calyx and carpel cavities, and the extending fissures were torn and fused with the sinus. **G,** Hyphae ramified vertically in the sinus toward the fruit core. **H,** Opened sinus extended to the carpel cavities. TT: transmitting tissues. Bars: A, C–E = 200 μm; F, G = 500 μm; B, H = 1 mm.

why core rot on 'Fuji' apple occurs at late growth stages, including the preharvest season.

The structure of calyx tubes of apple fruit also poses challenges for control of core rot. After bloom, some flower parts remain attached to the fruit surface and other parts are sealed inside of the calyx tube. It is reasonable to infer that spent flower parts inside the calyx tube would be shielded from fungicide sprays by the sepals. Therefore, fungicide spraying before calyx tubes become enclosed would probably be more efficient than spraying after they become enclosed, although this idea awaits experimental confirmation.

Since some flower tissues remain on fruit for extended periods after bloom, they continue to play important roles in the infection cycle of core rot. Chen et al. (2002) found that spent flower parts of many apple fruit were colonized by *T. roseum*. Our study showed that this pathogen infected apple floral organs and that it remained in calyx tubes after fruit setting. Therefore, reducing flower infections more effectively may be an important direction for improving control of core rot.

Based on these results, the following suggestions should be considered when developing more effective management strategies: 1) reduce infection rates of flower tissues as much as possible by spraying fungicides during or after bloom; 2) apply fungicides before the sepals enclose the core; and 3) develop biological control agents which can colonize flower parts and inhibit ramification of *T. roseum*.

Only a certain percentage of 'Fuji' fruit develop open sinuses, so some fruit exhibit only browning fissures but not core rot. However, it is possible that such fruit may develop core rot during postharvest storage. Further research is merited to assess the postharvest risk associated with infected fruit that are asymptomatic at harvest.

In this study, we showed that *T. roseum* can infect the fruit core through stylar fissures in unified styles. Other fruit diseases that can be caused stylar infections include gray mold on strawberry and red raspberry caused by *Botrytis cinerea* (Bristow et al. 1986; McNicol et al. 1985) and mummy berry disease on blueberry caused by *Monilinia vaccinii-corymbosi* (Ngugi and Scherm 2004). However, their hyphae ingress into fruit ovaries via transmitting tissues of styles, or stylar canals, sharing the same path with the pollen tubes. Pathogens can infect plants via natural openings, including stomata, hydathodes, lenticels, nectaries, and stylar canals. Our results showed that the stylar fissure is a newly recognized natural opening for pathogen infection.

Our study clarified the pathways by which *T. roseum*, the predominant core rot pathogen in China, exploited spent flower parts and the open-sinus anatomy of 'Fuji' fruit to cause core rot. Moldy core and core rot are pathogen complexes. How these causal agents cooperate or compete with each other in occupying the same ecological niche remains unclear. Fungal fluorescence labeling used in this research would be an effective technique for helping to answer these questions.

In China, besides its role as a core rot pathogen, *T. roseum* is also a causal agent of black spot on bagged 'Fuji' apple (Dai et al. 2019b). Most black spots occur on fruit pericarps surrounding the sepals. In the present study, we showed that *T. roseum* can infect most apple flower tissues. Therefore, we infer that inocula of black spot may originate from apple flower debris. In addition, propagules on black spots may be a source of inoculum for core rot.

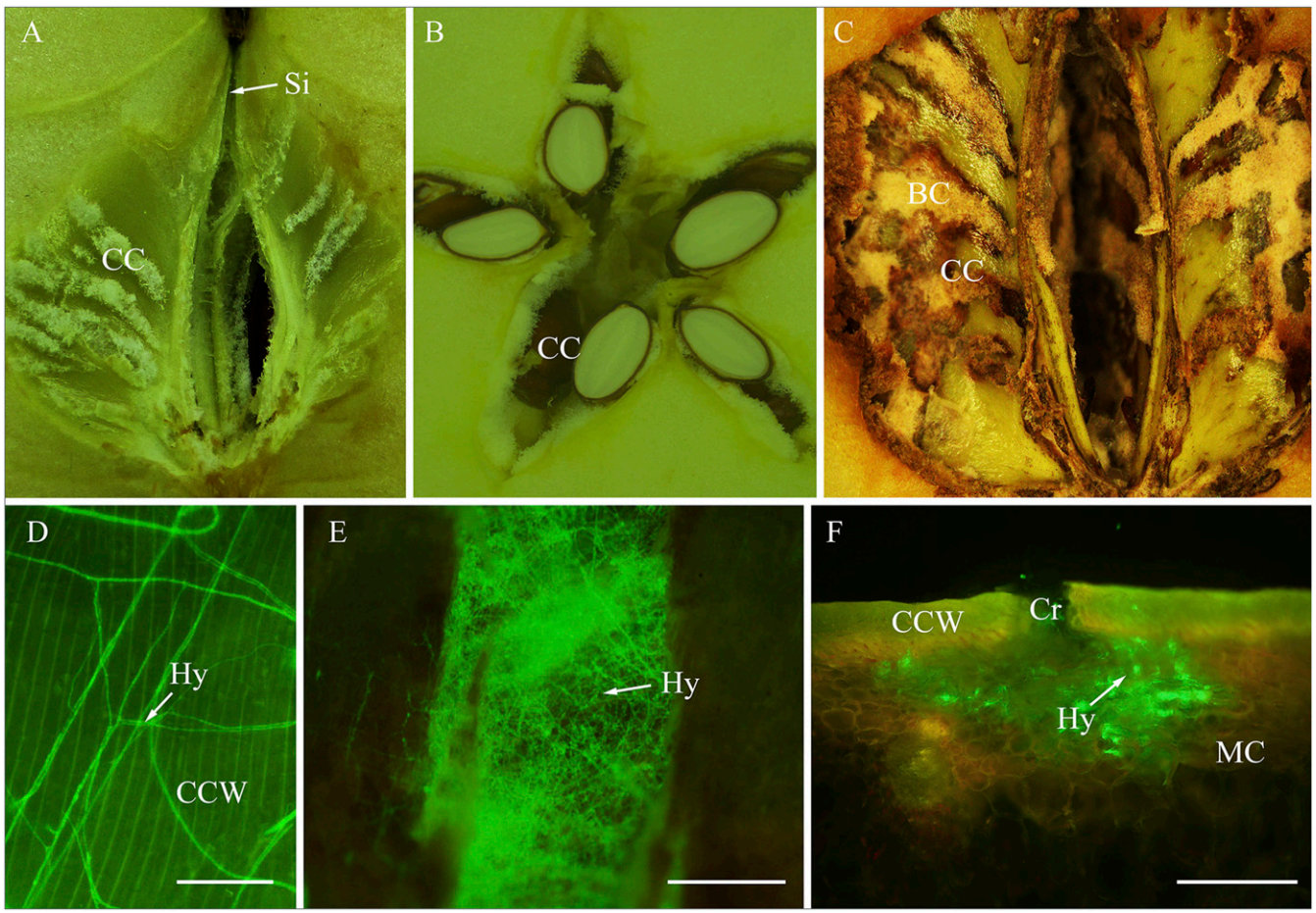

Fig. 8. Crack infection in carpel cavities. **A,** Carpel cavities (CC) were fused with the sinus (Si). **B,** Carpel cavities were connected and produced an open core (transverse section). **C,** Browning cracks (BC) in the carpel cavity of ripening fruit. **D,** Hyphae (Hy) ramified on carpel cavity walls (CCW). **E,** Hyphae proliferating at cracks beneath carpel cavity walls. **F,** Hyphae invading mesodermal cells (MC) via cracks (Cr). Bar: D = 50 μm; E, F = 200 μm.

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