Protoplasma
https://doi.org/10.1007/s00709-018-1254-9

ORIGINAL ARTICLE

Histological and cytological studies of plant infection by *Erysiphe euonymi-japonici*

Juanni Yao[1,2] · Dan Yu[1] · Yulin Cheng[1,2] · Zhensheng Kang[1]

Received: 26 January 2018 / Accepted: 16 April 2018

Abstract

Powdery mildew caused by *Erysiphe euonymi-japonici* (*Eej*) is an increasingly serious fungal disease on *Euonymus japonicus* that is an important ornamental plant. However, little is currently known about infection and pathogenesis of *Eej* on *E. japonicus*. Here, we report plant infection by *Eej* at the histological and cytological levels. *Eej* caused severe disease symptoms with white and snow-like colonies on leaf surfaces of *E. japonicus*. Microscopic observations were conducted continuously to define infection process of *Eej* on *E. japonicus*. *Eej* conidia germinated to produce appressorial germ tubes on leaf surfaces and formed irregular haustoria in plant epidermal cells at 6 h post-inoculation (hpi) and 12 hpi, respectively. After uptaking nutrients from host cells by haustoria, *Eej* formed numerous hyphae and extensive colonization on leaf surfaces at 96 hpi and finally produced abundant conidiophores and new conidia on leaf surfaces at 168 hpi. In addition, there was consistently a single nucleus in different *Eej* infection structures and haustorial development could be divided into three major stages, including formation of penetration peg, formation of haustorial neck and initial haustorium, and maturation of haustorium. These results provide useful information for further determination of *Eej* pathogenesis and finally controlling the disease.

Keywords *Euonymus japonicus* · *Erysiphe euonymi-japonici* · Powdery mildew · Microscope · Infection process

Introduction

As an important ornamental plant, *Euonymus japonicus* is widely planted in parks and landscapes of China and plays a significant role in urban and rural greening and environmental improvement (Huang et al. 2016). However, *E. japonicus* is often seriously damaged by many insects and microorganisms (Yi et al. 2002). Powdery mildew caused by *Erysiphe euonymi-japonici* (*Eej*) is one of deadly fungal diseases on *E. japonicus* (Li et al. 2011). In China, the increasingly serious disease (designated *Euonymus* powdery mildew) has occurred severe outbreaks recently (Li et al. 2011). In addition, *Euonymus* powdery mildew is also relatively common in Japan and also occurs in America and Europe (Boesewinkel 1980). A better understanding of how fungal pathogens infect and damage host plants is necessary for controlling diseases (Schafer 1994). Although surface and cellular structures of *Eej* conidiophores were observed (Li et al. 2011), little is currently known about infection and pathogenesis of *Eej* on *E. japonicus*.

As obligate biotrophic pathogens, powdery mildew fungi represent a large group of microorganisms and can cause diseases on thousands of plant species (Braun et al. 2002). Of them, *Blumeria graminis* has emerged as the model to study interactions of powdery mildew fungi and their hosts (Brown 2002; Zhang et al. 2005; Glawe 2008) and its infection process has been well researched (Lipka et al. 2008; Gan et al. 2012). After landing on a plant, asexual conidia attach to the plant surface and form two germ tubes, the primary germ tube and the appressorial germ tube. At the end of the appressorial germ tube, an appressorium is produced for host cell penetration. Upon entry, a specialized feeding structure, the haustorium, invaginates the host plasma membrane in living epidermal cells. Subsequent colonization together with conidia production indicates a successful infection and gives rise to

Juanni Yao and Dan Yu contributed equally to this work.

Handling Editor: Ulrike Mathesius

✉ Yulin Cheng
cyl02105026@163.com

✉ Zhensheng Kang
kangzs@nwsuaf.edu.cn

[1] State Key Laboratory of Crop Stress Biology for Arid Areas, Northwest A&F University, Yangling 712100, Shaanxi, China

[2] School of Life Sciences, Chongqing University, Chongqing, China

Published online: 25 April 2018

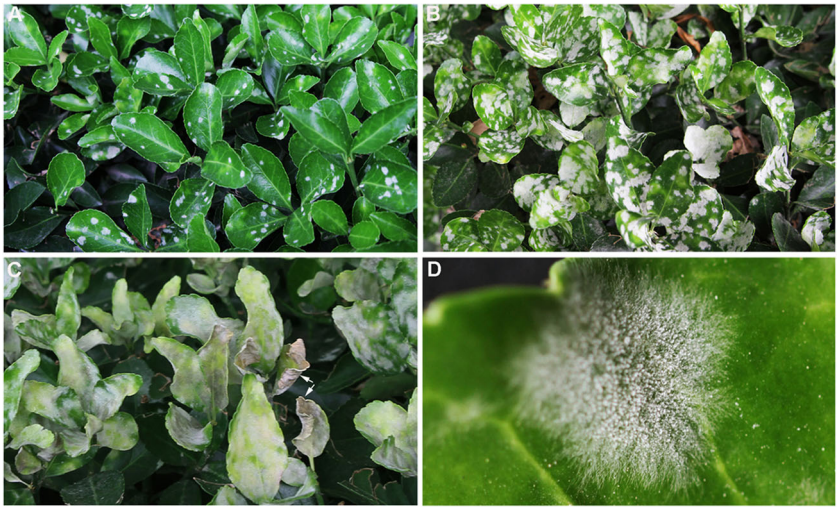

Fig. 1 Severe disease symptoms of *Euonymus japonicus* infected by *Erysiphe euonymi-japonici* (*Eej*). **a** Several white and snow-like patches were observed on leaf surfaces. **b** Patches extended to cover nearly whole leaf surfaces. **c** Some leaves (arrow) became chlorotic, curled, and withered. **d** The enlargement of patches

typical disease symptoms. It is worth mentioning that haustorium is a hallmark of obligate biotrophic pathogens and the key sites for absorption of nutrients essential for pathogen growth, development, and reproduction (Voegele and Mendgen 2011; Gan et al. 2012). However, *B. graminis* occurs only on grasses and differs significantly from other powdery mildew fungi (Braun et al. 2002; Inuma et al. 2007). There is only a limited understanding of plant infection by other powdery mildew fungi (Vogel and Somerville 2002). Thus, the study of plant infection by other powdery mildew fungi is of great significance to completely understand how powdery mildew fungi infect their hosts.

In this study, plant infection by *Eej* was investigated at the histological and cytological levels and our results indicate obvious differences in plant infection between *Eej* and well-known *B. graminis*. This work provides useful information for further determination of *Eej* pathogenesis and finally controlling the disease.

Materials and methods

Plants, pathogens, and inoculation

E. japonicus and *Eej* used in this study were collected from natural green areas in Northwest A&F University. For inoculation, health and fresh leaves of adult *E. japonicus* plants were collected with sharp scissors and cultured in a culture dish where 40 mg/L 6-benzylaminopurine was added to delay leaf senescence. Freshly collected *Eej* conidia (10^5 spores mL^{-1}) were applied with a fine paintbrush to the upper surface of *E. japonicus* leaves. Inoculated leaves in culture dishes were kept in a 25 °C growth chamber for 7 days, and each day 100% RH in the dark for 12 h followed by incubation at 60–70% RH for 12 h photoperiod (60 μmol m^{-2} s^{-1} photon flux density).

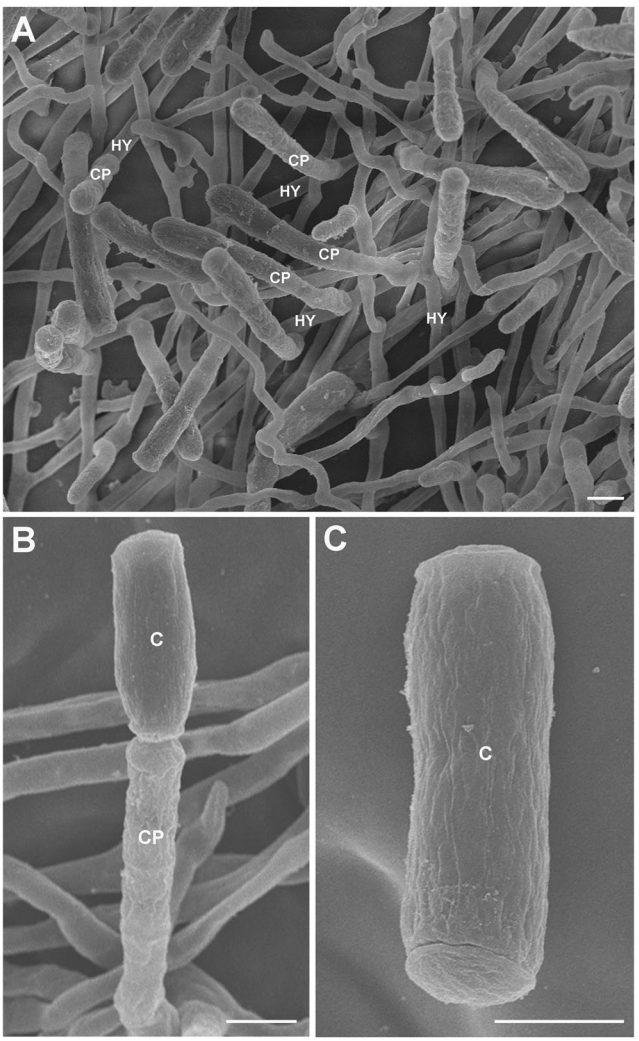

Fig. 2 Scanning electron microscopy observation of white and snow-like colonies of *Eej*. **a** Many hyphae (HY) and conidiophores (CP) were observed in the colonies. **b**, **c** The enlargement of conidiophore (CP) and conidium (C). Samples were taken from leaf surfaces of *E. japonicus*. Bar = 10 μm

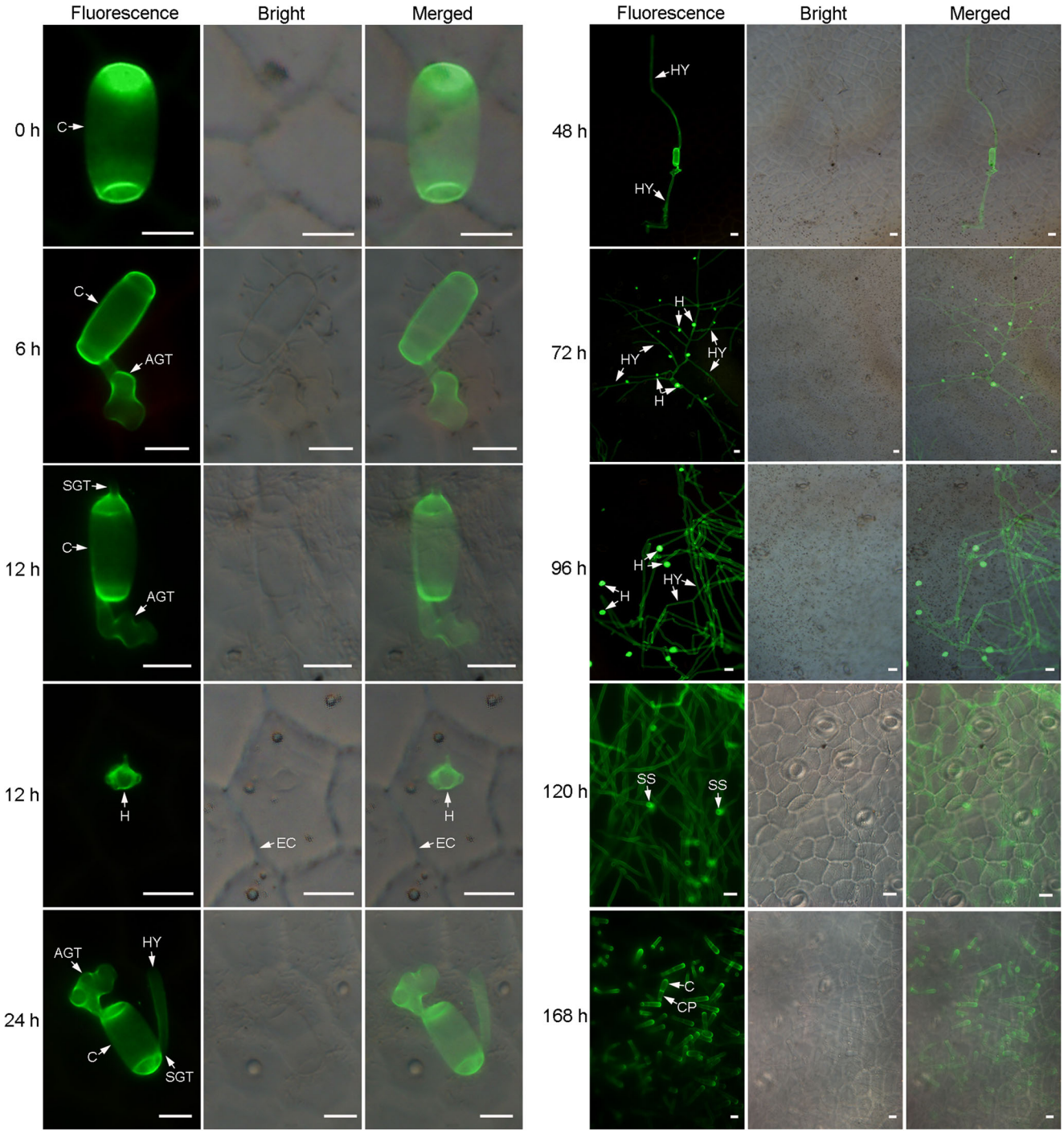

Fig. 3 Infection features of *Eej* on *E. japonicus* at a serial time points post-inoculation. Leaves were examined under an epifluorescence microscope after staining with WGA-alexa staining. *C*, conidium; *AGT*, appressorial germ tube; *SGT*, second germ tube; *H*, haustorium; *EC*, epidermal cell; *HY*, hyphae; *SS*, sporogenous structure; *CP*, conidiophore. Bar = 10 μm

Bright-field and fluorescence microscopy

Infected *E. japonicus* leaf pieces of 2–3 cm^2 were harvested at a series of continuous time points (0, 6, 12, 24, 48, 72, 96, 120, and 168 hpi). Three independent experiments were done and at least ten leaf pieces were analyzed at each time point for one independent experiment. Then, samples were fixed and decolorized in ethanol:trichloromethane (3:1, *v*/*v*) containing 0.15% (*w*/*v*) trichloroacetic acid for 5–7 days and cleared in saturated chloral hydrate as described previously (Cheng et al. 2012). To visualize fungal structures, samples were stained with wheat germ agglutinin (WGA) conjugated to fluorophore alexa 488 (Invitrogen, USA) as described previously (Ayliffe et al. 2011). Finally, stained samples were examined under the

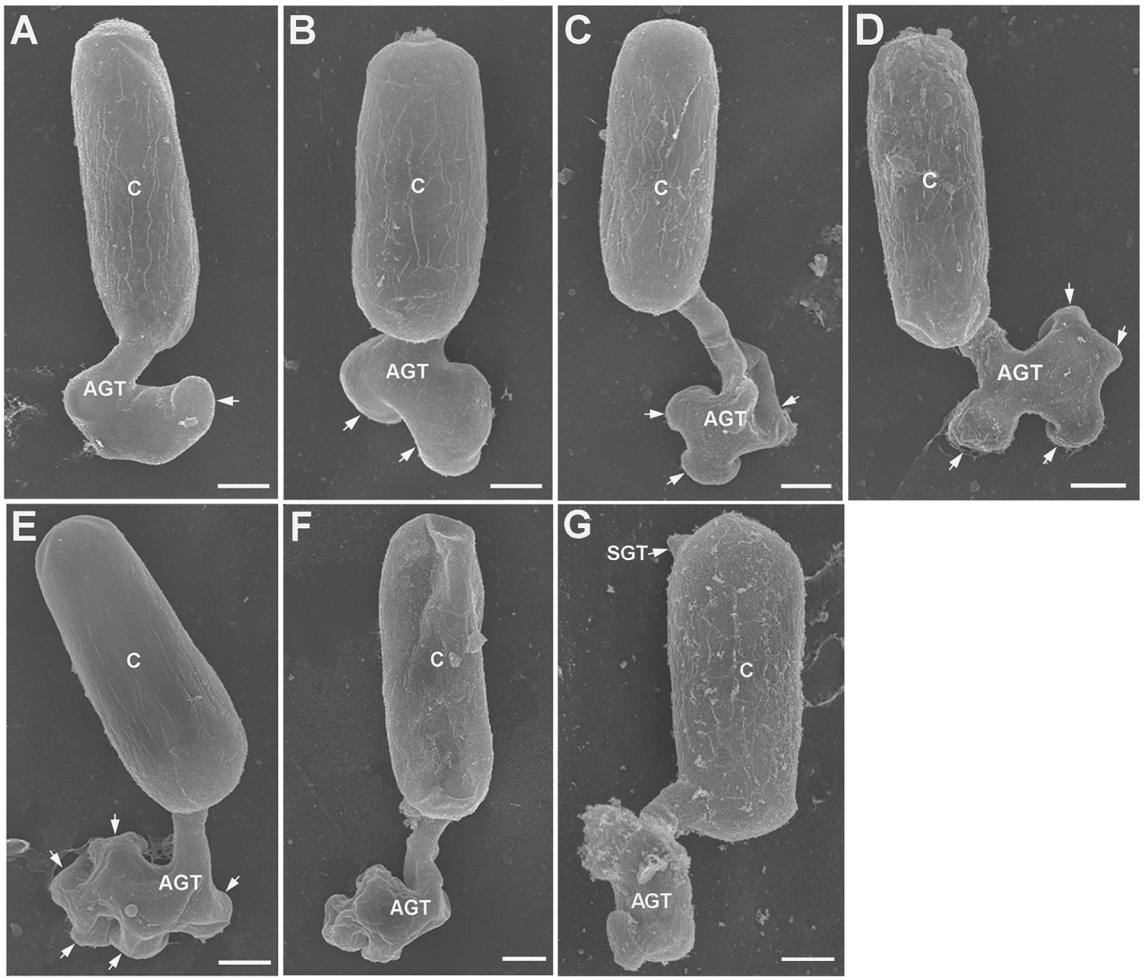

Fig. 4 Scanning electron microscopy observation of conidia germination of *Eej*. *C*, conidium; *AGT*, appressorial germ tube; *SGT*, second germ tube. Arrows in **a**–**f** indicate branches of AGT. Samples in **a**–**e** and **f**, **g** were taken at 6 h post-inoculation (hpi) and 12 hpi, respectively. Bar = 5 μm

fluorescent microscope Olympus BX-53 (Olympus Corporation, Japan) with excitation at 460–480 nm and emission at 495–540 nm.

Scanning electron microscopy

To observe surface structures of *Eej*, samples of scanning electron microscopy were done as described previously (Huang et al. 2008). Infected *E. japonicus* leaf pieces of 0.5–1 cm^2 were harvested, fixed in 4% (*v*/*v*) glutaraldehyde in 0.1 mol/L phosphate buffer (pH = 6.8) at 4 °C overnight, rinsed with the same buffer for 1–2 h again, and dehydrated in a graded series of ethanol. Then, samples were dried under a CO_2 atmosphere for 20 min using the critical point dryer K850 (Emitech, UK), sputtered with gold for 80 s using the sputter coater E1045 (Hitachi, Japan), and examined under a Hitachi S-4800 field emission scanning electron microscope (Hitachi, Japan) at a 10.0-kV voltage.

Transmission electron microscopy

To observe cellular structures of *Eej*, samples of transmission electron microscopy were done as described previously (Kang and Buchenauer 2000). *E. japonicus* leaf pieces of 0.2–0.5 cm^2 were harvested, fixed in 4% (*v*/*v*) glutaraldehyde in 0.1 mol/L phosphate buffer (pH = 6.8) at 4 °C overnight, and rinsed with the same buffer for 1–2 h again. Then, samples were fixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, infiltrated in 3:1, 1:1, and 1:3 (*v*/*v*) ethanol:white resin mixture (London resin company Ltd, England) and pure resin. In filtration, time in each mixture was at least 6 h and in pure resin 48 h. Samples were then embedded in capsules and polymerized at 55 °C for 2 days. Ultrathin sections of samples cut with a diamond knife were collected on copper grids. After contrasting with uranyl acetate and lead citrate, samples were examined using an HT-7700 transmission electron microscope (Hitachi, Japan) at an 80.0-kV voltage.

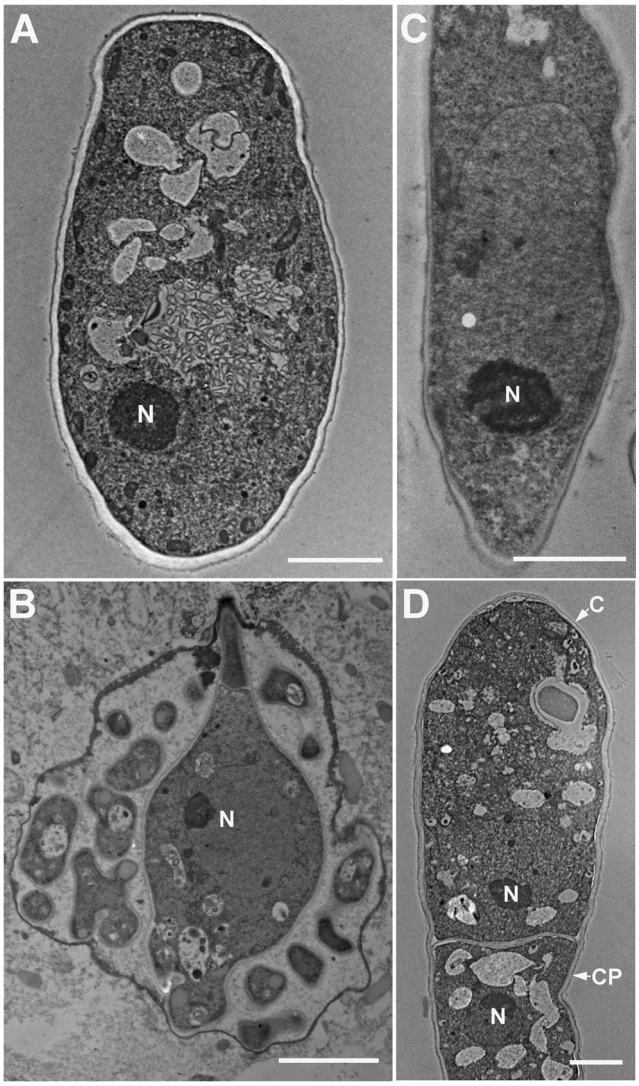

Fig. 5 Transmission electron microscopy observation of nucleus number in different infection structures of *Eej*. Infection structures include conidium (**a**), haustorium (**b**), hyphae (**c**), and conidiophore and newly generated conidium (**d**). *N*, nucleus; *CP*, conidiophore; *C*, conidium. Bar = 2 μm

Results

Severe disease symptoms of *Euonymus* powdery mildew

Several white and snow-like patches (Fig. 1a) were observed on leaf surfaces of *E. japonicus* infected by *Eej* in its early stages. The patches subsequently extended to cover nearly whole leaf surfaces (Fig. 1b) and infected *E. japonicus* leaves finally became chlorotic, curled, and withered (Fig. 1c). A large amount of white powder can be observed in the enlargement of patches (Fig. 1d), which is consistent with termed "*Euonymus* powdery mildew." Scanning electron microscopy observation of patches showed many hyphae and conidiophores (Fig. 2a), indicating that patches were *Eej* colonies. In addition, conidiophore was clavate and unbranched, and produced only one cylindrical conidium (Fig. 2b, c). These results show severe disease symptoms of *Euonymus* powdery mildew.

Infection process of *Eej* on *E. japonicus*

To determine infection process of *Eej* on *E. japonicus*, samples of infected *E. japonicus* were taken at a serial time points post-inoculation with *Eej* (Fig. 3). After landing on leaves of *E. japonicus*, *Eej* conidia attached to plant surfaces and germinated to produce appressorial germ tubes at 6 h post-inoculation (hpi) (Fig. 3). Noticeably, appressoria at the end of appressorial germ tubes were swollen and lobed with one to five branches (Fig. 4a–e). Conidia and appressorial germ tubes may be shrinking (Fig. 4f) with the development of *Eej* probably because of nutrient transport to other infection structures. At 12 hpi, a second, functional germ tube (Fig. 4g) emerged at the opposite end of conidium from the appressorial germ tube and began to form a haustorium in epidermal cells of *E. japonicas* (Fig. 3). The second germ tubes then produced hyphae at 24 hpi (Fig. 3). At 48 hpi, hyphae from both germ tubes elongated following the leaf surfaces of *E. japonicas* (Fig. 3). Each infection site of *Eej* produced more hyphae and haustoria at 72 hpi (Fig. 3). At 96 hpi, numerous hyphae formed a network on the leaf surface (Fig. 3). Sporogenous structures were formed at 120 hpi (Fig. 3). At 168 hpi, abundant conidiophores and new conidia were produced (Fig. 3), which indicates a completed infection of *Eej* on *E. japonicus*.

Nucleus number and haustorium development of *Eej* in the infection of *E. japonicus*

The change of nucleus number is important for taxonomy and genetic variation in fungi (Zhao et al. 2011) and some fungal pathogens have different nucleus numbers in different infection structures (Little and Manners 1969; Kang et al. 1993). However, transmission electron microscopy observations showed that there was consistently a single nucleus in different infection structures of *Eej*, including conidium (Fig. 5a), haustorium (Fig. 5b), hypha (Fig. 5c), and conidiophore (Fig. 5d).

In addition, haustorium development of *Eej* was also investigated by transmission electron microscopy. It can be divided into three major stages, including the formation of a penetration peg (Fig. 6a), formation of a haustorial neck and initial haustorium (Fig. 6b), and maturation of the haustorium (Fig. 6c). Noticeably, the haustorial neck did not pierce the plasma membrane of the plant cell (Fig. 6b, c) and one plant cell occasionally contains two haustoria (Fig. 6d). Mature haustoria were irregular with many plots and closely associated with plant cell nucleus (Fig. 6c, d).

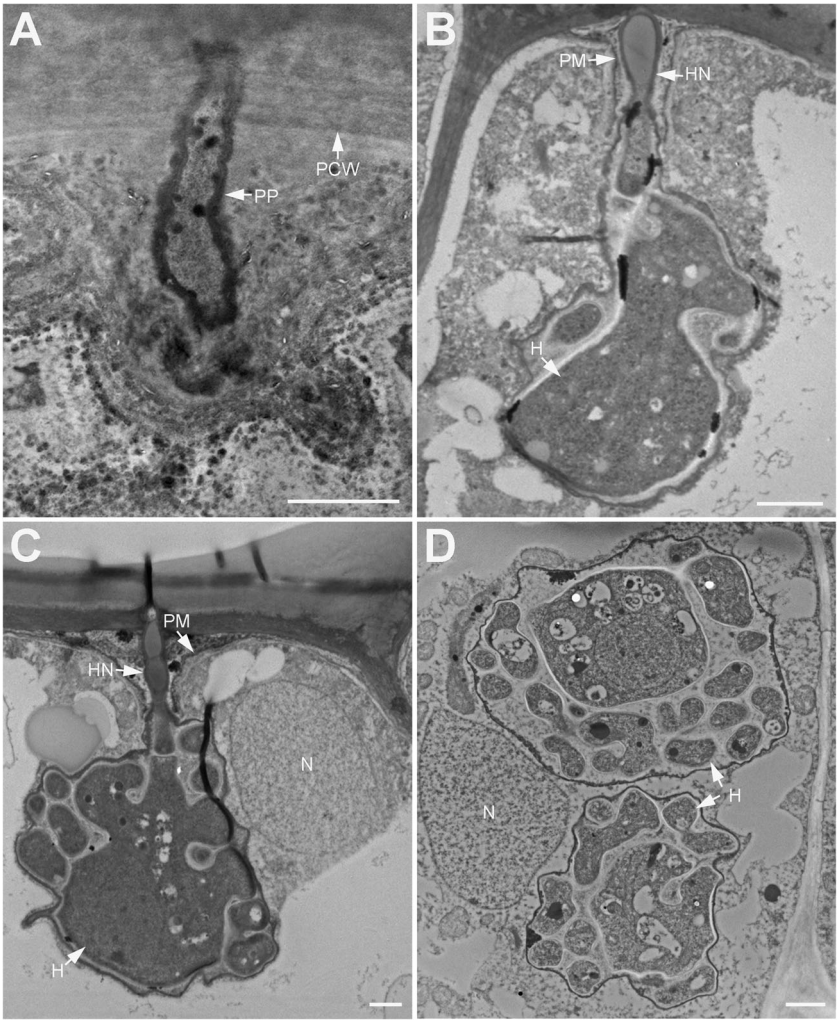

Fig. 6 Transmission electron microscopy observation of *Eej* haustorium development. **a** Penetration peg (PP) pierces plant cell wall (PCW). **b** Formation of haustorial neck (HN) and initial haustorium **(**H**)**. **c** Mature haustorium (H). **d** One plant cell contains two haustoria (H). *PM*, (plant) plasma membrane; *N*, (plant) nucleus. Samples in **a**, **b** were taken at 12 hpi, samples in **c** were taken at 24 hpi, and samples in **d** were taken at 48 hpi. Bar = 1 μm

Discussion

As an important fungal disease, *Euonymus* powdery mildew caused by *Eej* showed severe disease symptoms with white and snow-like colonies on leaf surfaces, which influences greatly production and ornamental value of *E. japonicus*. In this study, we described infection process, nucleus number, and haustorium development of *Eej* on *E. japonicus* at the histological and cytological levels, which is the first investigating into plant infection by *Eej*.

Understanding of fungal infection process is the key to know how fungal pathogens infect and damage host plants (Verhoeff 1980). Based on our results, the infection process of *Eej* can be summarized in Fig. 7. The infection process of

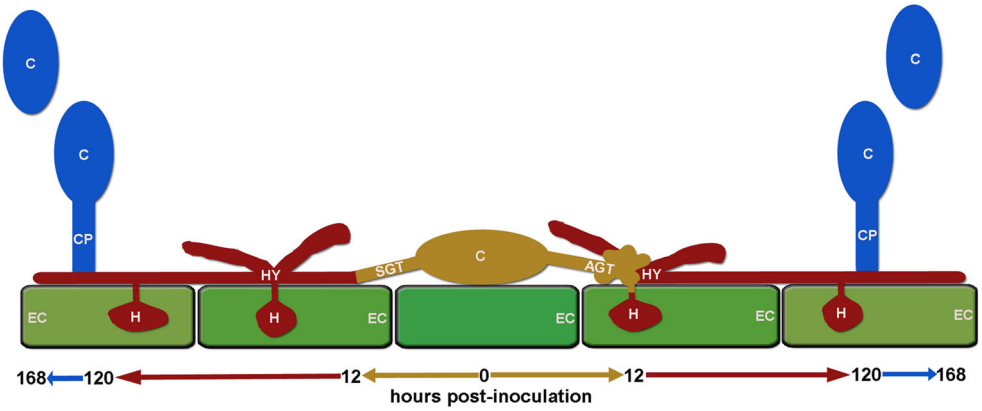

Fig. 7 Schematic representation of infection process of *Eej* on *E. japonicus*. It can be divided into three major phases, including "penetration stage" (yellow), "parasitic/biotrophic" (red), and "sporulation" (blue). *C* conidium; *AGT*, appressorial germ tube; *SGT*, second germ tube; *H*, haustorium; *HY*, hyphae; *CP*, conidiophore; *EC*, epidermal cell

rust fungi, such as *Puccinia striiformis*, can be divided into three major stages, including penetration stage, parasitic/biotrophic stage, and sporulation stage (Gan et al. 2012; Cheng et al. 2016). As another obligate biotrophic pathogen, *Eej* does the same and its infection process can also be divided into the three stages. In penetration stage, *Eej* conidia germinated to produce appressorial germ tubes and second germ tubes in succession. Upon establishment of haustoria in plant epidermal cells, *Eej* is no longer metabolically independent and enters the parasitic/biotrophic stage. After uptaking nutrients from host cells by haustoria, *Eej* formed numerous hyphae and extensive colonization on leaf surfaces. At the sporulation stage, *Eej* forms sporogenous structures and then produces abundant conidiophores and new conidia on leaf surfaces.

B. graminis is a model powdery mildew fungus and much of what we know about how powdery mildew fungi infect their hosts is based on this species (Glawe 2008). However, our results indicate obvious differences in plant infection between *Eej* and *B. graminis*. Firstly, *Eej* does not produce a primary germ tube but produces a second germ tube. A similar phenomenon was also observed in another powdery mildew fungus *E. cichoracearum* on *Arabidopsis* (Adam and Somerville 1996). The primary germ tube of *B. graminis* is believed to function in attaching germling and obtaining water or solutes from host cells (Carver and Bushnell 1983; Bélanger et al. 2002; Edwards 2002). The primary germ tube is absent in *Eej* and *E. cichoracearum*, indicating they have different and unknown mechanisms to attach and absorb. Secondly, *Eej* haustoria are irregularly shaped but *B. graminis* haustoria are digitate uniformly with many finger-like projections (Gan et al. 2012; Cheng et al. 2015). Finally, one conidiophore of *Eej* produces only one conidium but one conidiophore of *B. graminis* can produce chains of conidia (Zhang et al. 2005).

In addition, the mature haustorium of *Eej* was found to be closely associated with the cell nucleus of *E. japonicus*. A similar phenomenon was also observed in some rust fungi (Heath 1997) and the oomycete *Hyaloperonospora arabidopsidis* (Caillaud et al. 2012). The haustorium is thought to serve as a structure for delivery of virulence-associated "effector" proteins into plants (Panstruga and Dodds 2009; Godfrey et al. 2010; Bozkurt et al. 2011) and some effector proteins from oomycete pathogens are shown to target the host nucleus in order to re-program plant cell transcriptional machinery, suppress plant immune response, and subsequently promote pathogen infection (Caillaud et al. 2013). Increasing evidence indicates that close interaction between pathogen haustorium and plant cell nucleus is of great importance for plant infection by pathogens.

Overall, our results in this study answer how *Eej* infects *E. japonicus* at the histological and cytological levels. Considering the severity of *Euonymus* powdery mildew in ornamental plants, and obvious differences between *Eej* and well-known *B. graminis*, increasing availability of sequenced plant pathogen genomes which enables genomics-based discovery of pathogenicity-related genes (Martin and Kamoun 2011; Gibriel et al. 2016), and the gradually developed genetic transformation system of *E. japonicas* (Shang et al. 2008), *Eej* and *E. japonicas* may be also used as a model system to study plant pathogen interactions. Future work should be directed toward the investigation about which genes are required for *Eej* infection on *E. japonicus* and how *Eej* infects *E. japonicus* at the molecular level.

Funding information This study was supported by the National Natural Science Foundation of China (No. 31601597), the National Basic Research Program of China (No. 2013CB127700), and the Postdoctoral Science Foundation of Shannxi Province, China (No. 2016BSHTDZZ05).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adam L, Somerville SC (1996) Genetic characterization of five powdery mildew disease resistance loci in *Arabidopsis thaliana*. Plant J 9: 341–356. https://doi.org/10.1046/j.1365-313X.1996.09030341.x

Ayliffe M, Devilla R, Mago R, White R, Talbot M, Pryor A, Leung H (2011) Nonhost resistance of rice to rust pathogens. Mol Plant-Microbe Interact 24:1143–1155. https://doi.org/10.1094/MPMI-04-11-0100

Bélanger RR, Bushnell WR, Dik AJ, Carver TL (2002) The powdery mildews: a comprehensive treatise. Am Phytopathol Soc (APS Press)

Boesewinkel HJ (1980) The morphology of the imperfect states of powdery mildews (Erysiphaceae). Bot Rev 46:167–224. https://doi.org/10.1007/BF02860869

Bozkurt TO, Schornack S, Win J, Shindo T, Ilyas M, Oliva R, Cano LM, Jones AM, Huitema E, Van Der Hoorn RA (2011) *Phytophthora infestans* effector AVRblb2 prevents secretion of a plant immune protease at the haustorial interface. Proc Natl Acad Sci U S A 108: 20832–20837. https://doi.org/10.1073/pnas.1112708109

Braun U, Cook R, Inman A, Shin H (2002) The taxonomy of the powdery mildew fungi. The powdery mildews: a comprehensive treatise:13–55

Brown J (2002) Comparative genetics of avirulence and fungicide resistance in the powdery mildew fungi. The powdery mildews. a comprehensive treatise. APS Press, St. Paul, pp 56–65

Caillaud MC, Asai S, Rallapalli G, Piquerez S, Fabro G, Jones JD (2013) A downy mildew effector attenuates salicylic acid-triggered immunity in *Arabidopsis* by interacting with the host mediator complex. PLoS Biol 11:e1001732. https://doi.org/10.1371/journal.pbio.1001732

Caillaud MC, Piquerez SJ, Fabro G, Steinbrenner J, Ishaque N, Beynon J, Jones JD (2012) Subcellular localization of the Hpa RxLR effector repertoire identifies a tonoplast-associated protein HaRxL17 that confers enhanced plant susceptibility. Plant J 69:252–265. https://doi.org/10.1111/j.1365-313X.2011.04787.x

Carver T, Bushnell W (1983) The probable role of primary germ tubes in water uptake before infection by *Erysiphe graminis*. Physiol Plant Pathol 23:229–240. https://doi.org/10.1016/0048-4059(83)90005-X

Cheng Y, Wang W, Yao J, Huang L, Voegele RT, Wang X, Kang Z (2016) Two distinct Ras genes from *Puccinia striiformis* exhibit differential roles in rust pathogenicity and cell death. Environ Microbiol 18: 3910–3922. https://doi.org/10.1111/1462-2920.13379

Cheng Y, Yao J, Zhang H, Huang L, Kang Z (2015) Cytological and molecular analysis of nonhost resistance in rice to wheat powdery mildew and leaf rust pathogens. Protoplasma 252:1167–1179. https://doi.org/10.1007/s00709-014-0750-9

Cheng Y, Zhang H, Yao J, Wang X, Xu J, Han Q, Wei G, Huang L, Kang Z (2012) Characterization of non-host resistance in broad bean to the wheat stripe rust pathogen. BMC Plant Biol 12:96. https://doi.org/10.1186/1471-2229-12-96

Edwards H (2002) Development of primary germ tubes by conidia of *Blumeria graminis* f. sp. *hordei* on leaf epidermal cells of *Hordeum vulgare*. Can J Bot 80:1121–1125. https://doi.org/10.1139/b02-092

Gan PH, Dodds PN, Hardham AR (2012) Plant infection by biotrophic fungal and oomycete pathogens, in Signaling and communication in plant symbiosis. Springer 183–212

Gibriel HA, Thomma BP, Seidl MF (2016) The age of effectors: genome-based discovery and applications. Phytopathology 106:1206–1212. https://doi.org/10.1094/PHYTO-02-16-0110-FI

Glawe DA (2008) The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. Annu Rev Phytopathol 46:27–51. https://doi.org/10.1146/annurev.phyto.46.081407.104740

Godfrey D, Böhlenius H, Pedersen C, Zhang Z, Emmersen J, Thordal-Christensen H (2010) Powdery mildew fungal effector candidates share N-terminal Y/F/WxC-motif. BMC Genomics 11:317. https://doi.org/10.1186/1471-2164-11-317

Heath MC (1997) Signalling between pathogenic rust fungi and resistant or susceptible host plants. Ann Bot 80:713–720. https://doi.org/10.1006/anbo.1997.0507

Huang L, Buchenauer H, Han Q, Zhang X, Kang Z (2008) Ultrastructural and cytochemical studies on the infection process of *Sclerotinia sclerotiorum* in oilseed rape. J Plant Dis Protect 115:9–16. https://doi.org/10.1007/BF03356233

Huang L, Li QC, Zhang Y, Li DW, Ye JR (2016) *Colletotrichum gloeosporioides* sensu stricto is a pathogen of leaf anthracnose on evergreen spindle tree (*Euonymus japonicus*). Plant Dis 100:672–678. https://doi.org/10.1094/PDIS-07-15-0740-RE

Inuma T, Khodaparast SA, Takamatsu S (2007) Multilocus phylogenetic analyses within *Blumeria graminis*, a powdery mildew fungus of cereals. Mol Phylogenet Evol 44:741–751. https://doi.org/10.1016/j.ympev.2007.01.007

Kang Z, Buchenauer H (2000) Ultrastructural and immunocytochemical investigation of pathogen development and host responses in resistant and susceptible wheat spikes infected by *Fusarium culmorum*. Physiol Mol Plant Pathol 57:255–268. https://doi.org/10.1006/pmpp.2000.0305

Kang Z, Li Z, Shang H (1993) Nucleal condition of uredinial stage of wheat stripe rust. Acta Phytopathol Sin 24:26–31

Li C, Zhang Y, Liu Y, Kang J, Ma X, Fu L (2011) First report of powdery mildew caused by *Erysiphe euonymi-japonici* on *Euonymus japonicus* in central China. Plant Dis 95:611–611. https://doi.org/10.1094/PDIS-12-10-0942

Lipka U, Fuchs R, Lipka V (2008) *Arabidopsis* non-host resistance to powdery mildews. Curr Opin Plant Biol 11:404–411. https://doi.org/10.1016/j.pbi.2008.04.004

Little R, Manners J (1969) Somatic recombination in yellow rust of wheat (*Puccinia striiformis*): I. The production and possible origin of two new physiologic races. Trans Br Mycol Soc 53:251–258. https://doi.org/10.1016/S0007-1536(69)80059-8

Martin F, Kamoun S (2011) Effectors in plant-microbe interactions. John Wiley & Sons

Panstruga R, Dodds PN (2009) Terrific protein traffic: the mystery of effector protein delivery by filamentous plant pathogens. Science 324:748–750. https://doi.org/10.1126/science.1171652

Schafer W (1994) Molecular mechanisms of fungal pathogenicity to plants. Annu Rev Phytopathol 32:461–477. https://doi.org/10.1146/annurev.py.32.090194.002333

Shang A, Tian C, Zhao L, Tian Y (2008) Establishment of genetic transformation system of *Euonymus japonicus* 'Cu Zhi' mediated by *Agrobacterium tumefaciens*. Acta Horticulturae Sinica 35:409

Verhoeff K (1980) Infection process and host-pathogen interactions. Biology of *Botrytis*, edited by JR Coley-Smith, K. Verhoeff, WR Jarvis

Voegele RT, Mendgen KW (2011) Nutrient uptake in rust fungi: how sweet is parasitic life? Euphytica 179:41–55. https://doi.org/10.1007/s10681-011-0358-5

Vogel J, Somerville S (2002) Powdery mildew of *Arabidopsis*: a model system for host-parasite interactions. The powdery mildews: a comprehensive treatise. Belanger, RR, Bushnell, WR, Dik, AJ, Carver, TLW eds. The American Phytopathological Society Press. St. Paul 161–168

Yi Y, Li T, Ouyang J (2002) Species of important pests in *Euonymus japonicus* in western Hunan and their prevailing regularity. For Pest Dis 22:14–16

Zhang Z, Henderson C, Perfect E, Carver T, Thomas B, Skamnioti P, Gurr S (2005) Of genes and genomes, needles and haystacks: *Blumeria graminis* and functionality. Mol Plant Pathol 6:561–575. https://doi.org/10.1111/j.1364-3703.2005.00303.x

Zhao X, You W, Cui H, Yu X, Ye Z (2011) Studies on changes in nuclear conditions and molecule marker selection of polymorphism in *Ustilago esculenta*. J China Inst Metrol 22:176–180