An important role for secreted esterase in disease establishment of the wheat powdery mildew fungus *Blumeria graminis* f. sp. *tritici*

Jie Feng, Feng Wang, Geoff R. Hughes, Susan Kaminskyj, and Yangdou Wei

Abstract: The activity of esterase secreted by conidia of wheat powdery mildew fungus, *Blumeria graminis* f. sp. *tritici*, was assayed using indoxyl acetate hydrolysis, which generates indigo blue crystals. Mature, ungerminated, and germinating conidia secrete esterase(s) on artificial media and on plant leaf surfaces. The activity of these esterases was inhibited by diisopropyl fluorophosphate, which is selective for serine esterases. When conidia were inoculated on wheat leaves pretreated with diisopropyl fluorophosphate, both appressorial germ tube differentiation and symptom development were significantly impaired, indicating an important role of secreted serine esterases in wheat powdery mildew disease establishment.

Key words: serine esterase, cutinase, pathogenesis, powdery mildew, wheat.

Résumé : L'activité estérase sécrétée par les conidies de *Blumeria graminis* f. sp. *tritici*, le champignon responsable de l'oïdium (blanc) du blé, a été mesurée par l'hydrolyse de l'indoxyle acétate qui génère des cristaux bleu indigo. Les conidies matures, non germées, de même que les conidies en germination sécrètent des estérases sur du milieu artificiel et à la surface des feuilles des végétaux. L'activité de ces estérases était inhibée par le diisopropyle fluorophosphate, lequel est sélectif aux sérine estérases. Lorsque les conidies étaient inoculées sur les feuilles de blé prétraitées au diisopropyle fluorophosphate, la différenciation des tubes germinaux des appressoriums et le développement des symptômes étaient significativement réduits, ce qui indique que les sérine estérases secrétées jouent un rôle important dans l'établissement de l'oïdium chez le blé.

Mots-clés : sérine estérase, cutinase, pathogenèse, oïdium, blé.

[Traduit par la Rédaction]

Introduction

Disease establishment by obligate fungal plant pathogens requires successful host penetration so the pathogens can grow and reproduce. The plant cuticle, which is a nonspecific physical barrier external to the epidermis, plays an important role in the pathogenesis caused by direct-penetrating pathogens (Iwamoto et al. 2002; Zabka et al. 2008). The major components of the cuticle are cutins (esters of C_{16} or C_{18} fatty acids) and waxes (a complex mixture of alcohols, alkanes, aldehydes, ketones, and esters derived from long-chain fatty acids) (Kolattukudy 1985). Fungal esterase and cutinase, which hydrolyze cutins and waxes, are important for disease establishment by direct-penetrating pathogens. A better understanding of these enzymes could lead to the development of novel fungicides.

Serine esterases are a class of esterases in which a nucleophilic serine is the catalytic center of the enzyme. Among this class, members with serine located at the center of an extremely sharp turn between a β-strand and an α-helix are grouped into α/β hydrolase fold family of lipases (Longhi and Cambillau 1999). As members of this family, cutinases (EC 3.1.1.74) are specific for primary alcohol esters, the dominant linkage in cutin (Murphy et al. 1996). Cutinases differ from classical lipases (EC 3.1.1.3) in that they do not exhibit interfacial activation (Martinez et al. 1992). The catalytic triad at the active site of cutinase and other serine esterases, which consists of a serine, an aspartate, and a histidine (Petersen and Drabløs 1994), specifically interacts with chemical inhibitors, notably organophosphorus compounds such as diisopropyl fluorophosphate (DFP) (Dickman et al. 1982). This study focuses on the role(s) of secreted, nonspecific, DFP-inhibited serine esterases (hereafter termed esterases) produced by conidia and infection structures of *Blumeria graminis* f. sp. *tritici*.

Wheat and barley powdery mildew fungus (*Blumeria graminis* (DC.) Speer) penetrates host epidermal cells directly. Numerous studies have been conducted on the pathogenesis process, mainly focusing on *B. graminis* f. sp. *hordei*, the pathogenic agent on barley. Epidermal cell penetration by *B. graminis* f. sp. *hordei* may employ both enzymatic soft-

Received 21 September 2010. Revision received 30 November 2010. Accepted 3 December 2010. Published on the NRC Research Press Web site at cjm.nrc.ca on 17 February 2011.

J. Feng,[1] **F. Wang, S. Kaminskyj, and Y. Wei.**[2] Department of Biology, University of Saskatchewan, Saskatoon, SK S7N 5E2, Canada. **G.R. Hughes.** Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada.

[1]Present address: Crop Diversification Centre North, Edmonton, AB T5Y 6H3, Canada.
[2]Corresponding author (e-mail: yangdou.wei@usask.ca).

ening and mechanical force (Pryce-Jones et al. 1999). Both ungerminated and germinated conidia secrete hydrolytic enzymes, including esterase, cutinase, and lipase (Frič and Wolf 1994). Esterases are involved in spore adhesion (Kunoh et al. 1988), primary germ tube formation (Takahashi et al. 1985), and penetration (Frič and Wolf 1994) in barley powdery mildew pathogenesis.

Because of our interest in the cell-wall-degrading enzymes involved in the wheat – *B. graminis* f. sp. *tritici* pathosystem, we investigated the role of esterase(s) produced by *B. graminis* f. sp. *tritici* during infection. We demonstrated that *B. graminis* f. sp. *tritici* conidia and germlings secrete a DFP-inhibited esterase important for disease establishment on susceptible wheat.

Material and methods

All experiments were repeated at least twice with highly similar results. All chemicals were purchased from Fisher Scientific Canada (Ottawa, Ontario) unless otherwise specified.

Plant and fungal materials

Ten seeds of susceptible spring wheat (*Triticum graminis* L.) 'CDC Conway' were sown as a single row in 10 cm square pots. Seedlings were grown in a growth chamber set at 24 °C : 18 °C and a 16 h : 8 h (light:dark) photoperiod. The *B. graminis* f. sp. *tritici* strain was isolated in Saskatchewan as a single conidium from naturally infected CDC Conway. This strain is a typical of the Saskatchewan *B. graminis* f. sp. *tritici* population and was maintained on CDC Conway seedlings in the growth chamber as described above.

Seedling inoculation

Eleven days after seeding, the first leaves of all plants in a pot were immobilized on a horizontal plastic plate, abaxial side up, using 2 cotton strings. Leaves were inoculated by shaking mildewed plants above the seedlings in an inoculation box (Wei et al. 1998). By this method, the inoculation density can be controlled approximately 100 conidia per mm^2 of leaf surface. To ensure that only vigorous, young conidia were used in experiments, leaves bearing freely sporulating *B. graminis* f. sp. *tritici* colonies were shaken 48 h before inoculation to remove old conidia. Inoculated plants were maintained in the growth chamber as described above.

DFP treatment

Pure liquid DFP (EMD Biosciences, San Diego, California) was stored at –20 °C. DFP treatment solutions were prepared fresh for each experiment in 20 mmol/L Tris–HCl (pH 7.0) buffer containing 0.01% (*v*/*v*) Tween 20. DFP concentrations were 0.025, 0.25, and 2.5 mmol/L. The control treatment was 0.01% Tween 20 in 20 mmol/L Tris–HCl (pH 7.0).

For DFP treatments in planta, leaves were sprayed with DFP solution or Tris–Tween buffer, both at 5 mL/10 leaves, immediately after leaf immobilization but before inoculation. For spraying we used a 60 mL pump sprayer (Goody Products, Atlanta, Georgia) taking care to cover the whole leaf surface evenly. Plants were left for 1 h in the growth chamber, during which the surface dried completely, and were then inoculated with *B. graminis* f. sp. *tritici* conidia.

Infected leaves were sampled by removing the abaxial epidermis using fine forceps at 6 and 24 h after inoculation. In addition, 7 days after inoculation, infected leaves were photographed using a digital camera to assess disease intensity. Area measurements for disease rating used the ImageJ 1.34 software (http://rsb.info.nih.gov/ij).

Histochemical assay for surface localization of esterase activity

The qualitative esterase assay was modified from Rumbolz et al. (2000). The assay medium contained 3.4 mmol/L indoxyl acetate dissolved in 20 mmol/L Tris–HCl (pH 7.0) containing 0.99 mol/L NaCl, 44.6 mmol/L $CaCl_2$, and 17.5% gelatin. Glass microscope slides were coated with a 1 mm thick layer of assay medium, which was allowed to solidify. Slides were inoculated with *B. graminis* f. sp. *tritici* conidia at 100 conidia/mm^2, incubated at room temperature under continuous light, and examined 4 h after inoculation. Nonspecific esterase activity could be indicated by the presence of pigmented crystals of indigo blue, a product of hydrolysis of indoxyl acetate (Deising et al. 1992).

Cellular localization of esterase activity was determined by comparing unwashed *B. graminis* f. sp. *tritici* conidia and conidia that had been washed 5 times for 1 min each in 0.01% Tween 20 in 20 mmol/L Tris–HCl (pH 7.0). In addition, pieces of 7-day-infected wheat leaf bearing sporulating colonies were placed on assay slides to determine whether esterase was produced by conidia before secession. To test the inhibition of esterase activity by DFP, *B. graminis* f. sp. *tritici* conidia were inoculated on glass slides coated with assay medium containing 0.25 mmol/L DFP, then incubated and examined as above.

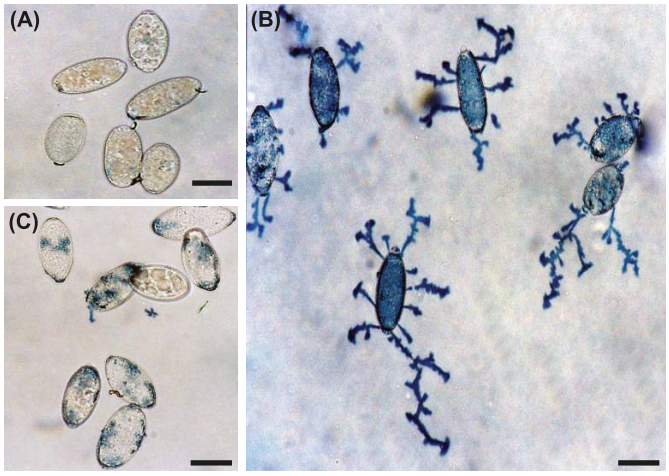
Microscopy

Epidermal peels and histochemical assays were examined by bright field microscopy, using a Zeiss Axiophot microscope (http://www.zeiss.com) and a 40× NA 1.25 objective. Images were captured using a SenSys digital camera (Photometrics, http://www.photometrics.com) driven by MetaVue software (http://www.metavue.com). To visualize conidia and haustoria, isolated epidermal peels were stained with Trypan blue before examination.

Results

After 4 h of incubation, no crystals were produced by the conidia on the assay medium without indoxyl acetate (Fig. 1A). In contrast, on the medium containing indoxyl acetate, conidia were surrounded by branched blue crystals (Fig. 1B), indicating the hydrolyzation of indoxyl acetate by esterase on the surface of the conidia (Deising et al. 1992). Around the washed conidia, no such crystals were produced (Fig. 1C), indicating that the esterase activity was extracellular or weakly bound to the spore surface because it could be removed by washing. Blue material (presumably tiny crystals) was also seen in the cytoplasm of unwashed and washed conidia. This is likely to be within lipid droplets in

Fig. 1. Qualitative assay of esterase activity of mature *Blumeria graminis* f. sp. *tritici* conidia using indoxyl acetate. After 4 h, esterase activity was shown by the presence of indigo blue crystals. (A) Conidia on assay media without indoxyl acetate. (B and C) Conidia on assay media with indoxyl acetate. (Panel B) Unwashed conidia are associated with long, branched indigo blue crystals. (Panel C) Washed conidia are not associated with indigo blue crystals, although there is minor staining in the cytoplasm. Bars represent 10 μm.



the conidia which can be mobilized, presumably by esterases, as a food resource during germination (Deising et al. 1992).

Esterase activity was assayed in planta for conidia borne on sporulating leaves (Fig. 2A). Under these conditions the indigo blue crystals were at least 2-fold longer at the top of conidial chains, suggesting that esterase abundance and (or) esterase activity increased as conidia matured. Indigo blue crystals were also produced in vitro by germinated conidia, as well as by primary and appressorial germ tubes (Fig. 2B). These observations indicated that mature *B. graminis* f. sp. *tritici* conidia can secrete esterase before and after secession as well as after germination.

The esterase secreted by *B. graminis* f. sp. *tritici* conidia was inhibited by serine esterase inhibitor DFP. After 4 h of incubation on assay media, conidia incubated in the absence of DFP were associated with abundant indigo blue crystals (Fig. 3A), whereas those assayed in media containing 0.25 mmol/L DFP were not (Fig. 3B). This result indicated that surface-localized esterases were of the serine esterase class. Moreover, the significant inhibition of esterase activity by 0.25 mmol/L DFP suggested that this concentration was sufficient for qualitative evaluation of *B. graminis* f. sp. *tritici* esterase activity.

Blumeria graminis f. sp. *tritici* conidia produced primary and appressorial germ tubes on untreated wheat leaves by 6 h after inoculation (Fig. 4A). In contrast, conidia on 0.25 mmol/L DFP-treated wheat leaves either did not germinate or produced only primary germ tubes (Fig. 4B). The numbers of ungerminated conidia, germinated conidia with only primary germ tube, and germinated conidia with both primary and appressorial germ tubes were counted under the microscope. Nearly 80% of conidia on untreated leaves produced appressorial germ tubes and more than 90% of conidia on treated leaves either failed to germinate or produced primary germ tubes only (Fig. 4C). Thus, it appeared that products released by esterase action on cuticle were important for stimulating germination and infection structure formation in *B. graminis* f. sp. *tritici*. Subsequently, *B. graminis* f. sp. *tritici* haustoria developed on untreated wheat leaves within 24 h after inoculation (Fig. 4D), whereas no haustoria were detected on DFP-treated leaves at 24 h after inoculation (data not shown).

Fig. 2. Localization of esterase activity on maturing conidia and during conidial germination using indoxyl acetate medium. (A) Conidia on an infected leaf 6 days after inoculation. Indigo blue crystals are longest on mature conidia at the top of conidial chains. (B) Production of a primary germ tube (PGT) and an appressorial germ tube (AGT) by a 4-h-old germling. Both are associated with indigo blue crystals. Bars represent 10 μm.

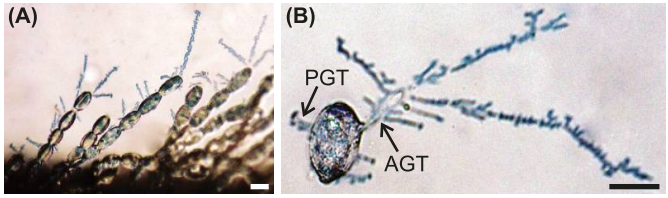
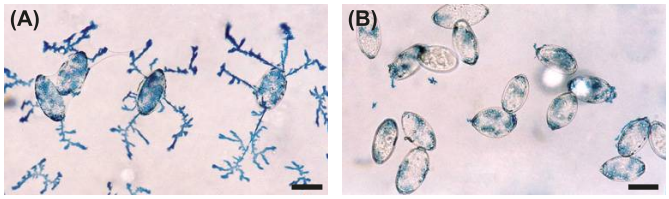


Fig. 3. Inhibition of *Blumeria graminis* f. sp. *tritici* esterase activity by diisopropyl fluorophosphate (DFP). Mature conidia were incubated on assay medium (A) lacking or (B) containing 0.25 mmol/L DFP. Bars represent 10 μm.

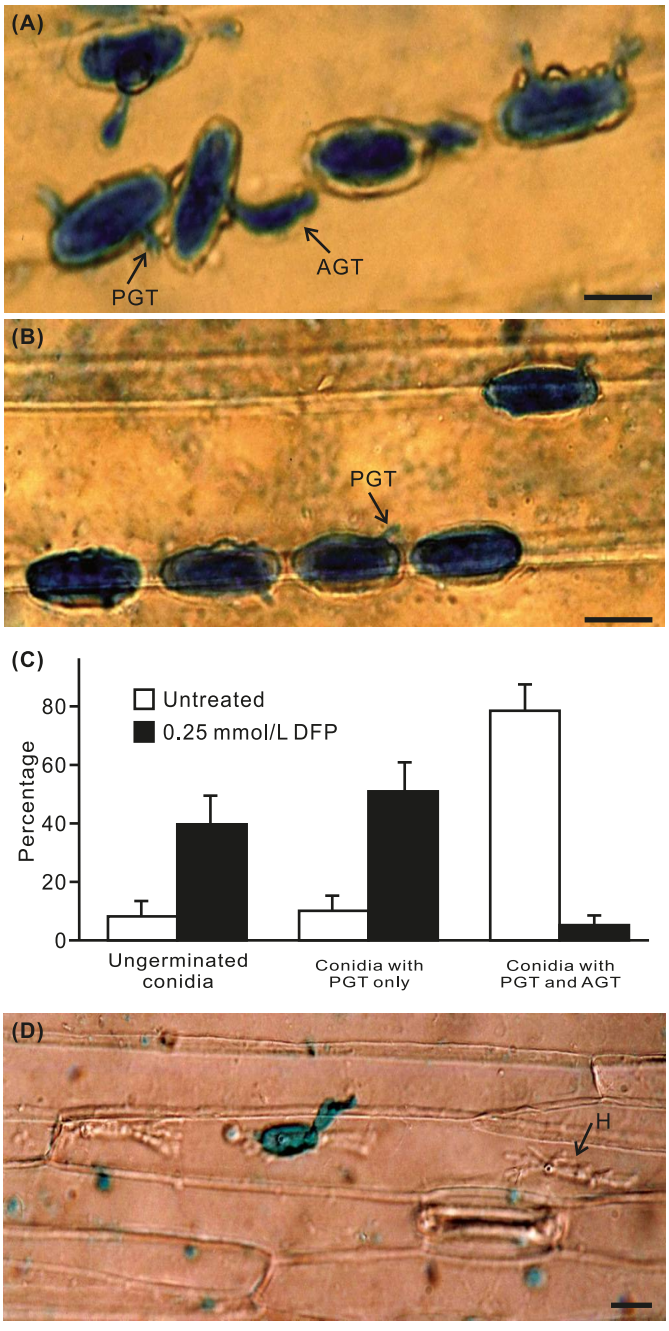


DFP treatment reduced *B. graminis* f. sp. *tritici* disease intensity in planta. Seven days after inoculation, extensive colonies developed on untreated leaves, whereas few colonies developed on DFP-treated leaves (Fig. 5). We defined the infection index as area of leaf with sporulating *B. graminis* f. sp. *tritici* / total leaf area. In Fig. 5, the untreated leaf has an infection index of 0.73 compared with infection indexes of 0.13, 0.07, and 0.02, respectively, for 0.025, 0.25, and 2.5 mmol/L DFP-treated leaves.

Discussion

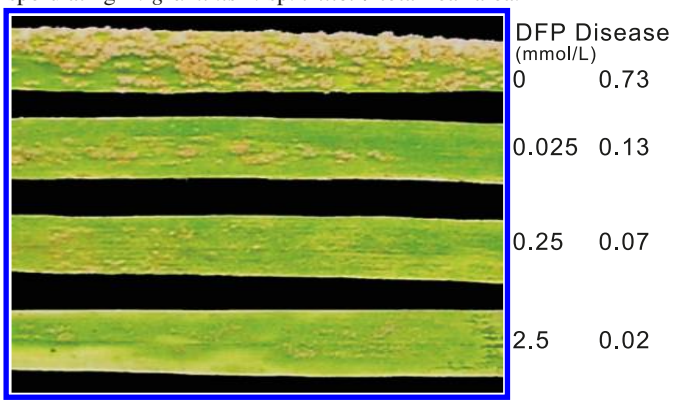
In this study, we have shown that DFP-sensitive esterase(s) have an important role in *B. graminis* f. sp. *tritici* disease establishment on susceptible wheat leaves. Similar results have been reported for other fungal pathosystems. Dickman et al. (1982) showed that DFP suppresses lesion formation by *Colletotrichum gloeosporioides* on papaya. Pascholati et al. (1993) reported that after treatment with DFP, the anthracnose fungus *Colletotrichum graminicola* fails to develop disease on corn leaves, despite normal appressorium formation. Using gene disruption, esterases such as cutinase (Rogers et al. 1994; Li et al. 2003; Skamnioti and Gurr 2007) and lipase (Voigt et al. 2005) have been demonstrated to be important for the pathogenicity of various plant pathogenic fungi.

Fig. 4. Effect of diisopropyl fluorophosphate (DFP) on *Blumeria graminis* f. sp. *tritici* conidial germination and appressorial development on susceptible wheat leaf surfaces 6 h after inoculation. (A) Control leaf. (B) Leaf pretreated with 0.25 mmol/L DFP. (C) On both control and DFP-treated leaves, ungerminated conidia, germinated conidia with only primary germ tubes (PGT), and germinated conidia with both primary and appressorial germ tubes (AGT) were counted within the microscope view (10 × 40) and plotted as the percentage against the total. The data were based on 5 countings on each of 10 leaves. (D) Development of haustoria (H) in control leaf epidermal cells at 24 h after inoculation. Bars represent 10 μm.



Contradictory evidence as to the importance of esterases in disease establishment has been proposed from an inhibitor study (Bonnen and Hammerschmidt 1989) and from disruption of individual esterase-encoding genes (Stahl and Schäfer 1992; Sweigard et al. 1992; Stahl et al. 1994; Crowhurst et al. 1997; van Kan et al. 1997; Reis et al. 2005), suggesting that these enzymes are not required for fungal pathogenicity. These conflicting interpretations may be due to characteristics of the pathosystem being studied and (or) they may be methodological. The roles of esterase likely vary in different pathosystems, so that no general model can be applied (Li et al. 2003). Also, some direct-penetrating pathogens, such as *Magnaporthe grisea*, rely on mechanical force only for penetration (Howard et al. 1991). Furthermore, most pathogenic fungi carry multiple genes likely encoding esterase activity, thus disruption of individual genes might fail to eliminate this (Annis and Goodwin 1997). Gene disruption methods are not yet well established for obligate biotrophs, such as powdery mildew fungi.

Fig. 5. Effect of diisopropyl fluorophosphate (DFP) on powdery mildew colony development 7 days after inoculation. Numbers indicate concentrations of DFP and infection index: area of leaf with sporulating *B. graminis* f. sp. *tritici* / total leaf area.



Pascholati et al. (1992) reported that esterase inhibition by DFP was optimal at pH 8.9 and reduced at pH 7.0. Nevertheless, we show significant inhibition of esterase activity and of *B. graminis* f. sp. *tritici* conidial germination by DFP at pH 7.0. DFP treatment of plant leaves at pH 9.0 led to extensive chlorosis at all DFP concentrations, as well as causing a dramatic reduction in disease after conidial inoculation. It is possible that the DFP concentrations on leaves treated at pH 7.0 compensated for lower activity due to pH effects.

We used DFP to inhibit esterase activity and found that 0.25 mmol/L DFP was sufficient to all but abolish *B. graminis* f. sp. *tritici* germination and differentiation. Inhibition of *B. graminis* f. sp. *tritici* appressorial germ tube differentiation by esterase inhibitors has been reported by Francis et al. (1996), who suggested that the cutin monomers may trigger appressorial germ tube differentiation. Our result can be explained by the same mechanism: DFP inhibits the release of cutin monomers by reducing esterase activity, which inhibits appressorial germ tube differentiation and, consequently, disease development. Since only a few appressorial germ tubes and very slight symptoms were detected on DFP-treated leaves, there appear to be no alternative mechanisms in the wheat – *B. graminis* f. sp. *tritici* pathosystem that can compensate for the secreted esterase. Thus, it appears that secreted esterase activity is exceedingly important for disease development in wheat powdery mildew. Final

proof of whether this is essential awaits development of reliable gene knockout procedures for obligate fungal biotrophs.

In this study, we did not further characterize whether the secreted esterase activity is contributed by lipase(s) or cutinase(s). However, a lipase has been identified from *B. graminis* f. sp. *tritici* and demonstrated to be important for spore adhesion (Feng et al. 2009). Whether the secreted esterase(s) identified from this study is actually lipase(s) needs further studies.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada and the Canadian Foundation for Innovation to the corresponding author.

References

Annis, S.L., and Goodwin, P.H. 1997. Recent advances in the molecular genetics of plant cell wall-degrading enzymes produced by plant pathogenic fungi. Eur. J. Plant Pathol. **103**(1): 1–14. doi:10.1023/A:1008656013255.

Bonnen, A.M., and Hammerschmidt, R. 1989. Role of cutinolytic enzymes in infection of cucumber by *Colletotrichum lagenarium.* Physiol. Mol. Plant Pathol. **35**(6): 475–481. doi:10.1016/0885-5765(89)90089-1.

Crowhurst, R.N., Binnie, S.J., Bowen, J.K., Hawthorne, B.T., Plummer, K.M., Rees-George, J., et al. 1997. Effect of disruption of a cutinase gene (*cutA*) on virulence and tissue specificity of *Fusarium solani* f. sp. *cucurbitae* race 2 toward *Cucurbita maxima* and *C. moschata.* Mol. Plant–Microbe Interact. **10**(3): 355–368. doi:10.1094/MPMI.1997.10.3.355. PMID:9100380.

Deising, H., Nicholson, R.L., Haug, M., Howard, R.J., and Mendgen, K. 1992. Adhesion pad formation and the involvement of cutinase and esterases in the attachment of uredospores to the host cuticle. Plant Cell, **4**(9): 1101–1111. doi:10.1105/tpc.4.9.1101. PMID:12297669.

Dickman, M.B., Patil, S.S., and Kolattukudy, P.E. 1982. Purification, characterization and role of infection of an extracellular cutinolytic enzyme from *Colletotrichum gloeosporioides* Penz. on *Carica papaya* L. Physiol. Plant Pathol. **20**(3): 333–347. doi:10.1016/0048-4059(82)90058-3.

Feng, J., Wang, F., Liu, G., Greenshields, D.L., Shen, W., Kaminskyj, S., et al. 2009. Analysis of a *Blumeria graminis*-secreted lipase reveals the importance of host epicuticular wax components for fungal adhesion and development. Mol. Plant–Microbe Interact. **22**(12): 1601–1610. doi:10.1094/MPMI-22-12-1601. PMID:19888825.

Francis, S.A., Dewey, F.M., and Gurr, S.J. 1996. The role of cutinase in germling development and infection by *Erysiphe graminis* f. sp. *hordei.* Physiol. Mol. Plant Pathol. **49**(3): 201–211. doi:10.1006/pmpp.1996.0049.

Frič, F., and Wolf, G. 1994. Hydrolytic enzymes of ungerminated and germinated conidia of *Erysiphe graminis* DC. f. sp. *hordei* Marchal. J. Phytopathol. **140**(1): 1–10. doi:10.1111/j.1439-0434.1994.tb00170.x.

Howard, R.J., Ferrari, M.A., Roach, D.H., and Money, N.P. 1991. Penetration of hard substrates by a fungus employing enormous turgor pressures. Proc. Natl. Acad. Sci. U.S.A. **88**(24): 11281–11284. doi:10.1073/pnas.88.24.11281. PMID:1837147.

Iwamoto, M., Takeuchi, Y., Takada, Y., and Yamaokaf, N. 2002. Coleoptile surface cuticle of barley is involved in survival and penetration of *Blumeria graminis.* Physiol. Mol. Plant Pathol. **60**(1): 31–38. doi:10.1006/pmpp.2001.0374.

Kolattukudy, P.E. 1985. Enzymatic penetration of the plant cuticle by fungal pathogens. Annu. Rev. Phytopathol. **23**(1): 223–250. doi:10.1146/annurev.py.23.090185.001255.

Kunoh, H., Yamaoka, N., Yoshioka, H., and Nicholson, R.L. 1988. Preparation of the infection court by *Erysiphe graminis*. I. Contact-mediated changes in morphology of the conidium surface. Exp. Mycol. **12**(4): 325–335. doi:10.1016/0147-5975(88)90024-2.

Li, D., Ashby, A.M., and Johnstone, K. 2003. Molecular evidence that the extracellular cutinase Pbc1 is required for pathogenicity of *Pyrenopeziza brassicae* on oilseed rape. Mol. Plant–Microbe Interact. **16**(6): 545–552. doi:10.1094/MPMI.2003.16.6.545. PMID:12795380.

Longhi, S., and Cambillau, C. 1999. Structure-activity of cutinase, a small lipolytic enzyme. Biochim. Biophys. Acta, **1441**(2–3): 185–196. PMID:10570246.

Martinez, C., De Geus, P., Lauwereys, M., Matthyssens, G., and Cambillau, C. 1992. *Fusarium solani* cutinase is a lipolytic enzyme with a catalytic serine accessible to solvent. Nature, **356**(6370): 615–618. doi:10.1038/356615a0. PMID:1560844.

Murphy, C.A., Cameron, J.A., Huang, S.J., and Vinopal, R.T. 1996. *Fusarium* polycaprolactone depolymerase is cutinase. Appl. Environ. Microbiol. **62**(2): 456–460. PMID:8593048.

Pascholati, S.F., Yoshioka, H., Kunoh, H., and Nicholson, R.L. 1992. Preparation of the infection court by *Erysiphe graminis* f. sp. *hordei*: cutinase is a component of the conidial exudate. Physiol. Mol. Plant Pathol. **41**(1): 53–59. doi:10.1016/0885-5765(92)90048-Z.

Pascholati, S.F., Deising, H., Leite, B., Anderson, D., and Nicholson, R.L. 1993. Cutinase and non-specific esterase activities in the conidial mucilage of *Colletotrichum graminicola.* Physiol. Mol. Plant Pathol. **42**(1): 37–51. doi:10.1006/pmpp.1993.1004.

Petersen, S.B., and Drabløs, F. 1994. A sequence analysis of lipases, esterases and related proteins. *In* Lipases: their structure, biochemistry and application. *Edited by* P. Woolley and S.B. Petersen. Cambridge University Press, New York. pp. 23–48.

Pryce-Jones, E., Carver, E., and Gurr, S.J. 1999. The roles of cellulose enzymes and mechanical force in host penetration by *Erysiphe graminis* f. sp. *hordei.* Physiol. Mol. Plant Pathol. **55**(3): 175–182. doi:10.1006/pmpp.1999.0222.

Reis, H., Pfiffi, S., and Hahn, M. 2005. Molecular and functional characterization of a secreted lipase from *Botrytis cinerea.* Mol. Plant Pathol. **6**(3): 257–267. doi:10.1111/j.1364-3703.2005.00280.x. PMID:20565655.

Rogers, L.M., Flaishman, M.A., and Kolattukudy, P.E. 1994. Cutinase gene disruption in *Fusarium solani* f. sp. *pisi* decreases its virulence on pea. Plant Cell, **6**(7): 935–945. doi:10.1105/tpc.6.7.935. PMID:8069105.

Rumbolz, J., Kassemeyer, H.H., Steinmetz, V., Deising, H.B., Mendgen, K., Mathys, D., et al. 2000. Differentiation of infection structures of the powdery mildew fungus *Unicinula necator* and adhesion to the host cuticle. Can. J. Bot. **78**(3): 409–421. doi:10.1139/cjb-78-3-409.

Skamnioti, P., and Gurr, S.J. 2007. *Magnaporthe grisea* cutinase2 mediates appressorium differentiation and host penetration and is required for full virulence. Plant Cell, **19**(8): 2674–2689. doi:10.1105/tpc.107.051219. PMID:17704215.

Stahl, D.J., and Schäfer, W. 1992. Cutinase is not required for fungal pathogenicity on pea. Plant Cell, **4**(6): 621–629. doi:10.1105/tpc.4.6.621. PMID:1392588.

Stahl, D.J., Theuekauf, A., Heitefuss, R., and Schäfer, W. 1994. Cutinase of *Nectria haematococca* (*Fusarium solani* f. sp. *pisi*) is not required for fungal virulence or organ specificity on pea. Mol. Plant–Microbe Interact. **7**(6): 713–725. doi:10.1094/MPMI-7-0713.

Sweigard, J.A., Chumley, F.G., and Valent, B. 1992. Disruption of a *Magnaporthe grisea* cutinase gene. Mol. Gen. Genet. **232**(2): 183–190. PMID:1557024.

Takahashi, K., Aist, J.R., and Isreal, H.W. 1985. Distribution of hydrolytic enzymes at barley powdery mildew encounter sites: implications for resistance associated with papilla formation in a compatible system. Physiol. Plant Pathol. **27**(2): 167–184. doi:10.1016/0048-4059(85)90065-7.

van Kan, J.A.L., van't Klooster, J.W., Wagemakers, C.A.M., Dees, D.C.T., and van der Vlugt-Bergmans, C.J.B. 1997. Cutinase A of *Botrytis cinerea* is expressed, but not essential, during penetration of gerbera and tomato. Mol. Plant–Microbe Interact. **10**(1): 30–38. doi:10.1094/MPMI.1997.10.1.30. PMID:9002270.

Voigt, C.A., Schäfer, W., and Salomon, S. 2005. A secreted lipase of *Fusarium graminearum* is a virulence factor required for infection of cereals. Plant J. **42**(3): 364–375. doi:10.1111/j.1365-313X.2005.02377.x. PMID:15842622.

Wei, Y., Zhang, Z., Andersen, C.H., Schmelzer, E., Gregersen, P.L., Collinge, D.B., et al. 1998. An epidermis/papilla-specific oxalate oxidase-like protein in the defence response of barley attacked by the powdery mildew fungus. Plant Mol. Biol. **36**(1): 101–112. doi:10.1023/A:1005955119326. PMID:9484466.

Zabka, V., Stangl, M., Bringmann, G., Vogg, G., Riederer, M., and Hildebrandt, U. 2008. Host surface properties affect prepenetration processes in the barley powdery mildew fungus. New Phytol. **177**(1): 251–263. PMID:17937760.