Effect of Flumorph on F-Actin Dynamics in the Potato Late Blight Pathogen *Phytophthora infestans*

Chenlei Hua, Kiki Kots, Tijs Ketelaar, Francine Govers, and Harold J. G. Meijer

First, second, fourth, and fifth authors: Laboratory of Phytopathology, and second and third authors: Laboratory of Cell Biology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands; and fourth author: Centre for BioSystems Genomics, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

Current address of C. Hua: Institute of Microbiology, Chinese Academy of Science, Beijing, 100101.

Accepted for publication 5 November 2014.

ABSTRACT

Hua, C., Kots, K., Ketelaar, T., Govers, F., and Meijer, H. J. G. 2015. Effect of flumorph on F-actin dynamics in the potato late blight pathogen *Phytophthora infestans*. *Phytopathology* 105:419-423.

Oomycetes are fungal-like pathogens that cause notorious diseases. Protecting crops against oomycetes requires regular spraying with chemicals, many with an unknown mode of action. In the 1990s, flumorph was identified as a novel crop protection agent. It was shown to inhibit the growth of oomycete pathogens including *Phytophthora* spp., presumably by targeting actin. We recently generated transgenic *Phytophthora infestans* strains that express Lifeact-enhanced green fluorescent protein (eGFP), which enabled us to monitor the actin cytoskeleton during hyphal growth. For analyzing effects of oomycides on the actin cytoskeleton in vivo, the *P. infestans* Lifeact-eGFP strain is an

excellent tool. Here, we confirm that flumorph is an oomycide with growth inhibitory activity. Microscopic analyses showed that low flumorph concentrations provoked hyphal tip swellings accompanied by accumulation of actin plaques in the apex, a feature reminiscent of tips of nongrowing hyphae. At higher concentrations, swelling was more pronounced and accompanied by an increase in hyphal bursting events. However, in hyphae that remained intact, actin filaments were indistinguishable from those in nontreated, nongrowing hyphae. In contrast, in hyphae treated with the actin depolymerizing drug latrunculin B, no hyphal bursting was observed but the actin filaments were completely disrupted. This difference demonstrates that actin is not the primary target of flumorph.

Additional keyword: oomyceticide.

Phytophthora infestans is a notorious plant pathogen that causes late blight on potato and tomato and was responsible for the Great Famine in Ireland in the mid-19th century. It is one of the more than 100 species in a genus that comprises exclusively plant pathogens (17). For managing *Phytophthora* diseases, farmers often make use of synthetic agrochemicals and spray their crops at regular intervals in order to prevent or reduce yield losses. For controlling potato late blight, for example, the number of sprays per growing season can reach up to 15 (12).

Phytophthora spp. resemble fungi in many ways: they show hyphal growth, disperse via spores and exploit similar strategies to infect plants. Yet, evolutionarily, they are not related to fungi (18). The genus *Phytophthora* belongs to the oomycetes, a stramenopile lineage in the supergroup Chromalveolates, whereas fungi are members of the supergroup Unikonta (5,19). This difference in origin is reflected in differences in structural and biochemical properties. Two prominent examples that relate to the sensitivity to agrochemicals are cell wall composition and ergosterol biosynthesis. Cell walls in fungi are mainly composed of chitin whereas *Phytophthora* spp. have cell walls that contain primarily cellulose and β -glucans (3,10,24). Hence, chitin synthase inhibitors such as the fungicide nikkomycin Z have no inhibitory effect on

Phytophthora spp. (18). The same holds for azole fungicides. They target the sterol biosynthesis pathway and, unlike fungi, *Phytophthora* spp. do not synthesize sterols (18,21). The lack of these common targets explains why many of the agrochemicals marketed as general fungicides are less effective in controlling diseases caused by *Phytophthora* spp. and other oomycetes, including downy mildews and *Pythium* spp. (8). Genome-wide analyses have shown that oomycetes have quite a number of genes encoding proteins with unique combinations of protein domains (4,22,25). These novel proteins are not found in other eukaryotes and could be exploited for finding novel drug targets in oomycetes. Although compounds with activity against oomycetes are often still classified as fungicides, it is more accurate to use the term oomycides (9) or oomyceticides.

In the 1990s, a novel systemic compound with the common name flumorph was introduced (20,27). The active ingredient of flumorph is 4-[3-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-1-oxo-2-propenyl] morpholine. It belongs to the carboxylic acid amides (CAAs) and was shown to act as a strong oomycide effective against certain *Peronospora* and *Phytophthora* spp. (13,26,28,29). In China, flumorph is registered as a crop protection agent and currently applied in several crops to control oomycete diseases. As yet, however, the mode of action of flumorph is unknown. In *Pseudoperonospora cubensis*, Zhu and coworkers (28) observed some cross-resistance of flumorph with dimethomorph, a structural CAA analog, and with iprovalicarb, a structurally unrelated CAA, suggesting an interference with cell wall synthesis (28). In another study, the same authors observed that treatment of *Phytophthora melonis* with flumorph resulted in impairment of polar growth and cell wall deposition and suggested that flumorph disrupts F-actin organization (29).

The actin cytoskeleton consists of filamentous actin (F-actin) polymers. The actin cytoskeleton is highly dynamic and continuously

Corresponding author: C. Hua; E-mail address: huacl@im.ac.cn

C. Hua and K. Kots contributed equally to this work.

*The e-Xtra logo stands for "electronic extra" and indicates that one supplementary figure and three supplementary videos are published online.

<http://dx.doi.org/10.1094/PHYTO-04-14-0119-R>
© 2015 The American Phytopathological Society

reorganizes. It is essential for many cellular processes, including cell motility and cell shape changes during the cell cycle or in response to extracellular stimuli in vertebrate and invertebrate cells (15). This dynamicity of the actin cytoskeleton has been observed in several eukaryotes but, until recently, not in oomycetes. Thus far, the studies in oomycetes were mainly based on phalloidin staining of actin in hyphae or sporangia, with only static images as result. In a recent study, Meijer and coworkers analyzed the dynamics of the actin cytoskeleton in *P. infestans* in vivo by introducing a DNA fragment encoding the actin binding peptide Lifeact-enhanced green fluorescent protein (eGFP) into *P. infestans* (23). In transgenic Lifeact-eGFP strains, cortical actin filament plaques and actin filament cables were visualized in vivo and over time without affecting hyphal growth and virulence. The study of Meijer et al. (23) further revealed that, in contrast to actin filament patches in fungi, the actin filament plaques in *P. infestans* are highly immotile, are not internalized prior to their disassembly, and are relatively insensitive to actin depolymerization. As an experimental set-up, flow cells were used for microscopic observation of living hyphae. With this set-up, it is easy to expose the hyphae to drugs and immediately analyze the effect of the drug treatment in vivo with the microscope. In this way, the effect of latrunculin B (latB), a drug that affects free actin polymerization by binding actin monomers, was monitored (23). Thus, the Lifeact-eGFP strains are instrumental for visualizing in vivo changes in the actin cytoskeleton.

In this study, we exploited the *P. infestans* Lifeact-eGFP strains to investigate the mode of action of flumorph and to test the hypothesis

put forward by Zhu et al. (29) that the actin cytoskeleton is the primary target of flumorph in *P. infestans*.

MATERIALS AND METHODS

***P. infestans* strains and cultivation.** *P. infestans* strains 88069-mRFP (14) and Lifeact-eGFP (23) were maintained on rye sucrose agar (RSA) plates supplemented with geneticin at 5 µg/ml, as described (23).

Flumorph and latB solutions. A stock solution of 10 mM latB (Sigma-Aldrich, Zwijndrecht, The Netherlands) was prepared in dimethyl sulfoxide and diluted to the desired concentration in rye sucrose liquid (RS) medium, as previously described (16). A stock solution of flumorph (generously provided by J. Liu, Shenyang Research Institute of Chemical Industry, Shenyang, China) at 1 mg/ml was prepared in methanol (30) and diluted in RS medium to the desired concentration.

Flumorph sensitivity assays. Growth assays were performed in triplicate on RSA medium in six-well plates (CELL STAR, Greiner bio-one). A 5-mm-diameter fresh mycelial plug was inoculated in each well and the six-well plates were incubated at 18°C in the dark. The mycelial growth rate was determined by measuring the diameter of mycelial colony on RSA with and without flumorph each day after inoculation. The colony outgrowth was determined by comparing colony diameters of each strain on flumorph plate to that on nonflumorph plate at 6 days post-inoculation (dpi). The statistical data analysis was performed using Student's *t* test. The concentration that resulted in 50% growth reduction (EC₅₀) was determined based on the data points after 6 dpi.

Sample preparation for microscopy. Samples for microscopic imaging using either biofoil slides or flow cell slides were prepared as described previously (23). Germinating sporangia were incubated in the presence of latB or flumorph for the time indicated and then transferred to biofoil slides. To study the immediate effect of flumorph on actin organization, flow cells containing germinating sporangia were prepared before flumorph was added.

Microscopy and data analysis. *P. infestans* hyphae were monitored using a Roper (Evry, France) Spinning Disc Confocal System on a Nikon Eclipse Ti microscope using a ×100 Plan apo oil immersion objective (NA 1.4) and a 491-nm laserline. Optical sections were collected with 0.5 µm Z intervals. The images were taken by Metamorph software (<http://www.moleculardevices.com/products/software/meta-imaging-series/metamorph.html>) and processed using ImageJ software (<http://rsbweb.nih.gov/ij/>).

RESULTS AND DISCUSSION

Flumorph inhibits growth and stimulates sporangial development of *P. infestans* strain 88069. To determine the sensitivity to flumorph of the *P. infestans* strains used in this study, the mycelial growth rate was measured. In the absence of flumorph, no significant differences in growth were observed between strain 88069 expressing Lifeact-eGFP (hereafter named Lifeact-eGFP) and control strain 88069-mRFP (Fig. 1A). The 88069-mRFP strain expresses monomeric red fluorescent protein (mRFP) that was shown not to interfere with cellular processes (23). The result of this sensitivity assay suggests that, at the expression level we used, the actin binding capacity of Lifeact does not interfere with normal functioning of the actin cytoskeletal during growth and confirms previous observations (23). Adding flumorph to concentrations of up to 0.25 µg/ml did not affect the mycelial growth on agar medium. However, in the presence of flumorph at 0.5 µg/ml, a 10.2 ± 4.0% growth reduction was observed and, for flumorph at 0.75 µg/ml, a 50% growth reduction was reached. This EC₅₀ value of 0.75 µg/ml was significantly higher than the EC₅₀ values reported for other *P. infestans* strains, which were in the range of 0.10 to 0.33 µg/ml (26). We are not aware of any reports describing the flumorph sensitivity of *P. infestans* strain 88069, the recipient strain of the two

Fig. 1. Growth and morphology of a *Phytophthora infestans* Lifeact-eGFP strain in the presence and absence of flumorph. **A**, Relative colony sizes of *P. infestans* strains 88069-mRFP or Lifeact-eGFP on six-well plates containing rye sucrose agar (RSA) medium without additives (set at 100%) or in the presence of flumorph at 0.1, 0.25, 0.5, and 1 µg/ml (* indicates *P* < 0.05). **B**, Morphology of 88069-mRFP and Lifeact-eGFP hypha grown on RSA without additives (Control) and with flumorph at 1 µg/ml (Flumorph) or 750 nM latB (LatB) visualized by light microscopy. Recipient strain 88069 showed a similar morphology. Photos were taken from 7-day-old colonies. Scale bar is 200 µm and representative for all images.

transformants used in this study. It is possible that these differences in flumorph sensitivity are caused by strain- or medium-specific effects, a phenomenon that has been described before in sensitivity tests of *P. infestans* for certain compounds (2,26). When analyzing the mycelium of the Lifeact-eGFP and 88069-mRFP strain (Fig. 1B) by microscopy, we observed that flumorph at high concentrations (1 $\mu\text{g/ml}$) provoked aerial outgrowth of numerous escaping hyphae carrying multiple sporangia. This abnormal morphology might explain the observations made by Yuan et al. (26) that flumorph-resistant *P. infestans* strains produce fewer sporangia than sensitive strains. The morphological aberrations were not observed when the mycelium was exposed to actin-depolymerizing drug latB (Fig. 1B) and, hence, it is unlikely that actin depolymerization is causing the change in morphology.

Flumorph affects *P. infestans* morphology but not the actin cytoskeleton. Previously, Zhu et al. (29) described how flumorph treatment of *P. melonis* resulted in tip-swelling morphology and disruption of F-actin organization. For visualization of the actin cytoskeleton, they used phalloidin staining and confocal microscopy. We first monitored the hyphae with abnormal morphology during flumorph treatment for the 88069-mRFP control strain and the Lifeact-eGFP transformant. The EC₅₀, determined from solid RSA medium assays, was selected as flumorph concentration in liquid medium although the sensitivity of hyphae could differ between solid and liquid medium. Within 30 min after treatment with flumorph at 0.75 $\mu\text{g/ml}$, 50% of hyphae showed tip swelling ($n = 70$). These swelling tips showed at least 40% increase of tip diameter compared with the original hyphal diameter. The availability of a *P. infestans* Lifeact-eGFP strain allowed us to monitor the effect of flumorph on hyphal morphology in much more detail and, more intriguingly, to visualize the actin cytoskeleton dynamics in the same hyphae during flumorph treatment. In this set-up, we compared the effects induced by flumorph with those induced by treatment with latB, a known actin depolymerizing drug that, similar to flumorph, causes hyphal tip swelling (16,23). Our previous studies showed that the consequences of latB treatment are concentration dependent (16,23). At low concentrations, most

hyphae stopped growing and developed apical actin plaques (Fig. 2D–E; Supplementary Video S2). At intermediate concentrations, there was an initial total loss of actin cables and plaques but, within minutes, plaques reappeared. At high concentrations, all actin cytoskeletal structures were permanently lost but cytoplasmic streaming (Fig. 2F) and tip swelling were still observed (23). Similar to latB, flumorph caused a severe reduction of growth speed (Fig. 1A). Within 30 min after the addition of flumorph at 0.75 $\mu\text{g/ml}$ in many hyphae, plaques appeared up to the apical zone while maintaining the actin cytoskeleton structures (Fig. 2G and H). The presence of plaques in the apical area of hyphae is reminiscent of nongrowing hyphae (Fig. 2C). Plaque recruitment was often accompanied by swelling of the hyphal tip (Figs. 2G and H; 3C; Supplementary Video S3). This swelling is characteristic for *Phytophthora* isolates exposed to flumorph and might be due to an effect on the cell wall (29). We tested for local changes in cell wall composition induced by flumorph treatment or by Calcofluor white staining but did not detect an obvious difference in the amount or accessibility of cellulose in the cell wall at the hyphal tip (Supplementary Fig. S1). After overnight exposure to flumorph at 0.75 $\mu\text{g/ml}$, many hyphae had regained growth. These hyphae were devoid of plaques in the apical zone (Fig. 2I and J) and, in that respect, they resembled growing hyphae in nontreated cultures (Fig. 2A and B; Supplementary Video S1). In contrast, the nonapical parts of the hyphae contained many plaques (Fig. 2J), reflecting an actin plaque organization known from nongrowing hyphae (Fig. 2C). When hyphae were treated with flumorph at 1 $\mu\text{g/ml}$, rapid tip swelling was more prominent. A time series of a hyphal tip that responded within 2 min after exposure to flumorph at 1 $\mu\text{g/ml}$ is shown in Figure 3C. The rapid tip swelling was accompanied by recruitment of actin plaques into the apical zone, as clearly observed at the later time points (Fig. 3C; Supplementary Video S3). In addition to hyphae with swollen tips, hyphae with burst tips were also occasionally observed, in particular at higher flumorph concentrations. An example is shown in Figure 3D where exposure to flumorph at 1.5 $\mu\text{g/ml}$ resulted in a loss of fluorescence, accompanied with bursting of the hyphal tip. This differs from latB

Fig. 2. Actin cytoskeleton organization and hyphal morphology of the *Phytophthora infestans* Lifeact-eGFP strain under various conditions visualized by spinning disk confocal microscopy. **A**, Hypha growing in rye sucrose liquid (RS) medium without treatment with a relative fast growth speed of 155.4 $\mu\text{m/h}$. **B**, Hypha growing in RS medium without treatment with a relative slow growth speed of 57.1 $\mu\text{m/h}$. **C**, Nongrowing hypha in RS medium without treatment. **D** and **E**, Hyphae treated for 30 min with 200 nM latB showing tip swelling. **F**, Hypha treated with 10 μM latB. The picture was taken at 3 s after latB was added. **G** and **H**, Hyphae treated for 30 min with flumorph at 0.75 $\mu\text{g/ml}$. **I** and **J**, Hyphae treated overnight with flumorph at 0.75 $\mu\text{g/ml}$. Bar is 5 μm .

treatment, where we observed that the fluorescence signal was not lost (Fig. 2F) and apparently the cytoplasmic streaming was not disrupted (23). Because latB depolymerizes actin, the loss of fluorescence cannot be considered as a hallmark for the disruption of actin structures. We hypothesize that the burst hyphae die, which causes a rapid drop in eGFP fluorescence, possibly due to protein degradation or a drop in pH. Hyphae that display arrested growth during flumorph treatment regain growth after time. The actin filament plaques and actin filament cables shown in Figures 2 and 3 were repeatedly observed in three independent experiments. Treatment with flumorph did not change the organization of the actin cytoskeleton. All actin filament plaques and actin filament cables behaved as under control conditions, with the exception of a slight reduction in fluorescence levels. Aberrant actin structures, such as induced during latB treatment of hyphae (23), were not observed.

In summary, no notable effects of flumorph on the actin cytoskeleton organization in *P. infestans* were detected. The actin cytoskeleton in the flumorph-treated hyphae is reminiscent of that

in nongrowing, untreated hyphae. At high concentrations of flumorph there was a loss of fluorescence, which was accompanied with bursting of the hyphal tip. This is in contrast to the effect of latB where, at high concentrations, there was still fluorescence but this was dispersed throughout the cytoplasm and not confined to actin cytoskeleton structures due to their depolymerization (23). This difference suggests that, instead of disrupting the actin cytoskeleton, flumorph induces hyphal death, frequently accompanied by bursting of the hyphal tip. Based on our results, we conclude that flumorph inhibits the growth of *P. infestans* and, thus, is effective as an oomycete for late blight control. However, its mode of action is still unknown and its primary target remains to be elucidated.

Conclusions. In this study, we used a Lifeact-eGFP *P. infestans* marker strain to investigate the anticipated mode of action of the novel agrochemical flumorph. Flumorph was suggested to act by interfering with the actin cytoskeleton in *Phytophthora* spp. (29). However, under the conditions used in this study, no such interference was observed. Our results illustrate that marker strains

Fig. 3. Time series for actin cytoskeleton dynamics and hyphal development of the *Phytophthora infestans* Lifeact-eGFP strain under various conditions visualized by spinning disk confocal microscopy. **A**, Time series of a hypha growing in rye sucrose liquid (RS) medium without treatment (control condition). **B**, Time series of a hypha treated with 200 nM latB. Time point 1 shows the hypha 1 min after latB addition. **C**, Hypha treated with flumorph at 0.75 µg/ml. Time point 1 shows the hypha at the moment of flumorph addition. **D**, Hypha bursting after addition of flumorph at 1.5 µg/ml. Images were taken 40 min after addition of flumorph, with 8-s time interval, and show the actin cytoskeleton organization directly before bursting. The bright-field image shows the hyphal tip 2 min after bursting. Bars are 5 µm.

which allow real-time monitoring of vital signs such as the actin dynamics are powerful tools for characterizing the effect of (new) agrochemicals at the cellular level. In our microscopic analyses of *P. infestans* hypha treated with flumorph, we frequently observed hyphal bursting, which suggests that flumorph acts by interfering with the cell wall. The cell wall in oomycetes has a different composition than cell walls in other organisms (11,24) and, therefore, could be an interesting target for oomycides. In fact, the recently developed drug mandipropamid was found to inhibit an enzyme involved in cell wall biosynthesis in *P. infestans* (6). Generated CAA fungicide-resistant *P. melonis* strains were found to share a mutation in a cellulose synthase gene, suggesting that this compound also has a cell wall biosynthesis-influencing mode of action (7). Apart from the Lifeact-eGFP marker strain, various other *P. infestans* marker strains have been generated (1) but they remain to be exploited as tools for analyzing the mode of action of oomycides and guiding the development of new compounds. The availability of additional *P. infestans* marker strains (for example, to study the effects on the cell wall) can support the discovery of new, species-specific compounds with a known mode of action.

ACKNOWLEDGMENTS

This work was supported by the Dutch Technology Foundation STW-NWO in the framework of a VIDI project (H. J. G. Meijer). We thank J. Liu, Shenyang Research Institute of Chemical Industry, Shenyang, China, for providing flumorph.

LITERATURE CITED

- Ah-Fong, A. M., and Judelson, H. S. 2011. Vectors for fluorescent protein tagging in *Phytophthora*: Tools for functional genomics and cell biology. *Fungal Biol.* 115:882-890.
- Atuszak, J. M., Fernandez-Elquezabal, J., Gu, W. K., Villarreal-Gonzalez, M., and Fry, W. E. 1994. Sensitivity of *Phytophthora infestans* population to metalaxyl in Mexico: Distribution and dynamics. *Plant Dis.* 78: 911-916.
- Badreddine, I., Lafitte, C., Heux, L., Skandalis, N., Spanou, Z., Martinez, Y., Esquerre-Tugaye, M. T., Bulone, V., Dumas, B., and Bottin, A. 2008. Cell wall chitosaccharides are essential components and exposed patterns of the phytopathogenic oomycete *Aphanomyces euteiches*. *Eukaryot. Cell* 7:1980-1993.
- Bakthavatsalam, D., Meijer, H. J., Noegel, A. A., and Govers, F. 2006. Novel phosphatidylinositol phosphate kinases with a G-protein coupled receptor signature are shared by *Dictyostelium* and *Phytophthora*. *Trends Microbiol.* 14:378-382.
- Baldauf, S. L. 2003. The deep roots of eukaryotes. *Science* 300: 1703-1706.
- Blum, M., Boehler, M., Randall, E., Young, V., Csukai, M., Kraus, S., Moulin, F., Scalliet, G., Avrova, A. O., Whisson, S. C., and Fonne-Pfister, R. 2010. Mandipropamid targets the cellulose synthase-like PiCesA3 to inhibit cell wall biosynthesis in the oomycete plant pathogen, *Phytophthora infestans*. *Mol. Plant Pathol.* 11:227-243.
- Chen, L., Zhu, S., Lu, X., Pang, Z., Cai, M., and Liu, X. 2012. Assessing the risk that *Phytophthora melonis* can develop a point mutation (V1109L) in CesA3 conferring resistance to carboxylic acid amide fungicides. *PLoS One* 7:e42069.
- Cohen, Y., and Coffey, M. D. 1986. Systemic fungicides and the control of oomycetes. *Annu. Rev. Phytopathol.* 24:311-338.
- Govers, F. 2001. Misclassification of pest as 'fungus' puts vital research on wrong track. *Nature* 411:633.
- Grenville-Briggs, L. J., Anderson, V. L., Fugelstad, J., Avrova, A. O., Bouzenzana, J., Williams, A., Wawra, S., Whisson, S. C., Birch, P. R., Bulone, V., and van West, P. 2008. Cellulose synthesis in *Phytophthora infestans* is required for normal appressorium formation and successful infection of potato. *Plant Cell* 20:720-738.
- Grenville-Briggs, L. J., Avrova, A. O., Hay, R. J., Bruce, C. R., Whisson, S. C., and van West, P. 2010. Identification of appressorial and mycelial cell wall proteins and a survey of the membrane proteome of *Phytophthora infestans*. *Fungal Biol.* 114:702-723.
- Hijmans, R. J., Forbes, G. A., and Walker, T. S. 2000. Estimating the global severity of potato late blight with GIS-linked disease forecast models. *Plant Pathol.* 49:697-705.
- Hu, J. Y., Liu, C., and Yan, H. 2008. Degradation of flumorph in soils, aqueous buffer solutions, and natural waters. *J. Agric. Food Chem.* 56: 8574-8579.
- Hua, C., Meijer, H. J., de Keijzer, J., Zhao, W., Wang, Y., and Govers, F. 2013. GK4, a G-protein-coupled receptor with a phosphatidylinositol phosphate kinase domain in *Phytophthora infestans*, is involved in sporangia development and virulence. *Mol. Microbiol.* 88:352-370.
- Ketelaar, T. 2013. The actin cytoskeleton in root hairs: All is fine at the tip. *Curr. Opin. Plant Biol.* 16:749-756.
- Ketelaar, T., Meijer, H. J. G., Spiekerman, M., Weide, R., and Govers, F. 2012. Effects of latrunculin B on the actin cytoskeleton and hyphal growth in *Phytophthora infestans*. *Fungal Genet. Biol.* 49:1014-1022.
- Kroon, L. P., Brouwer, H., de Cock, A. W., and Govers, F. 2012. The genus *Phytophthora* anno 2012. *Phytopathology* 102:348-364.
- Latijnhouwers, M., de Wit, P. J., and Govers, F. 2003. Oomycetes and fungi: Similar weaponry to attack plants. *Trends Microbiol.* 11:462-469.
- Lee, S. C., Ristaino, J. B., and Heitman, J. 2012. Parallels in intercellular communication in oomycete and fungal pathogens of plants and humans. *PLoS Pathog.* 8:e1003028.
- Liu, W. C., Li, Z. L., Zhang, Z. J., and Liu, C. L. 2000. Antifungal activity and prospect of flumorph and its mixtures. *Zhejiang Chem. Ind.* 31:87-88.
- Madoui, M. A., Bertrand-Michel, J., Gaulin, E., and Dumas, B. 2009. Sterol metabolism in the oomycete *Aphanomyces euteiches*, a legume root pathogen. *New Phytol.* 183:291-300.
- Meijer, H. J. G., and Govers, F. 2006. Genomewide analysis of phospholipid signaling genes in *Phytophthora* spp.: Novelty and a missing link. *Mol. Plant-Microbe Interact.* 19:1337-1347.
- Meijer, H. J. G., Hua, C., Kots, K., Ketelaar, T., and Govers, F. 2013. Actin dynamics in *Phytophthora infestans*; rapidly reorganizing cables and immobile, long-lived plaques. *Cell. Microbiol.* 16:948-961.
- Meijer, H. J. G., van de Vondervoort, P. J., Yin, Q. Y., de Koster, C. G., Klis, F. M., Govers, F., and de Groot, P. W. 2006. Identification of cell wall-associated proteins from *Phytophthora ramorum*. *Mol. Plant-Microbe Interact.* 19:1348-1358.
- Seidl, M. F., Van den Ackerveken, G., Govers, F., and Snel, B. 2011. A domain-centric analysis of oomycete plant pathogen genomes reveals unique protein organization. *Plant Physiol.* 155:628-644.
- Yuan, S., Liu, X., Si, N., Dong, J., Gu, B., and Jiang, H. 2006. Sensitivity of *Phytophthora infestans* to flumorph: In vitro determination of baseline sensitivity and the risk of resistance. *Plant Pathol.* 55:258-263.
- Zhang, T., Zhao, Q., Zhang, Y., and Ning, J. 2013. Assessment of genotoxic effects of flumorph by the comet assay in mice organs. *Hum. Exp. Toxicol.* 33:224-229.
- Zhu, S., Liu, P., Liu, X., Li, J., Yuan, S., and Si, N. 2008. Assessing the risk of resistance in *Pseudoperonospora cubensis* to the fungicide flumorph in vitro. *Pest Manag. Sci.* 64:255-261.
- Zhu, S. S., Liu, X. L., Liu, P. F., Li, Y., Li, J. Q., Wang, H. M., Yuan, S. K., and Si, N. G. 2007. Flumorph is a novel fungicide that disrupts micro-filament organization in *Phytophthora melonis*. *Phytopathology* 97: 643-649.
- Zhu, S. S., Liu, X. L., Wang, Y., Wu, X. H., Liu, P. F., Li, J. Q., Yuan, S. K., and Si, N. G. 2007. Resistance of *Pseudoperonospora cubensis* to flumorph on cucumber in plastic houses. *Plant Pathol.* 56:967-975.