Eur J Plant Pathol (2014) 139:343–352
DOI 10.1007/s10658-014-0390-0

Erwinia persicina, a possible new necrosis and wilt threat to forage or grain legumes production

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Accepted: 16 January 2014 / Published online: 7 February 2014

Abstract *Erwinia persicina* (*Ep*) is a phytopathogenic bacteria found on *Medicago sativa*, *Glycine max*, *Phaseolus vulgaris* and *Pisum sativum*. It has also been isolated from healthy tomato, cucumber, banana, apple and pear, as well as the human urinary tract and biofilms located on paleolithic rock paintings. The host range and environmental adaptation capacity of *Ep*, and its life cycle on the lucerne plant, was studied. It was found that an *Ep* infection, which could be transmitted by seeds, water, and soil, caused necrosis and wilting of the whole mature lucerne plant. Subsequently, 15 genera and 22 species of forage or grain legumes were chosen to determine the pathogenicity of *Ep*. Among them were 11 genera and 19 species (e.g., *Vigna angularis*, *Arachis hypogaea*, *Onobrychis viciaefolia*, *Astragalus adsurgens,* etc.) reported as new hosts. The environmental adaptation capacity of *Ep* indicated its probable survival within a wide range of environmental conditions and an ability to endure arid, saline and alkaline environments. Based on the results of the life cycle, host range and environmental adaptation capacity of *Ep*, it was concluded that *Ep* could pose a new threat to forage or grain legumes production within farming systems.

Keywords *Erwinia persicina* · Environmental adaptation · Host range · Legumes · Life cycle

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Introduction

The legume plant family, Leguminosae, is one of the most popular crop families in the world (Frame 2001). Grain legumes, which include beans, peas, soy, peanuts, and lentils are especially important as a meat replacement because of their high nutritional profile, being excellent sources of protein and B vitamins, in addition to carbohydrates. Legumes lag behind only cereal or grain crops in terms of popularity as human food sources, and play an important role in global agriculture (Hymowitz 1990).

The benefits of forage legumes have been propounded mainly in terms of their contribution to the N economy of grasslands through N_2-fixation and their superior feeding value in relation to grasses (Frame 2001). Forage legumes, including lucerne and clover, are arguably the most important crops in the world. They are not only used as forage crops for feeding livestock, but also play a key role in improving soil fertility, controlling soil and water erosion, and reducing the negative effects of climate change (Zhang and Nan 2013). This, in return, supports the realization of food security, sustainable development and poverty reduction worldwide (Zhang and Nan 2013).

Erwinia persicinus is a bacterial species of the family *Enterobacteriaceae*, which forms pink-pigmented colonies and was first isolated from healthy tomato, banana, and cucumber (Hao et al. 1990). *E. persicinus* was shown to be distinct from all other validly published *Erwiniae*, including the pink-pigmented species, *E. rhapontici* (Brenner et al. 1994) and *E. rubrifaciens*

(Wilson et al. 1967). In 1994, Brenner et al. (1994) reported that *E. persicinus* was a senior subjective synonym for "*E. nulandii*", an organism that was pathogenic on bean (*Glycine max*) pods and seeds. In 1998, O'Hara et al. (1998) first isolated this phytopathogen from the human infected urinary tract. That same year, *E. persicinus* was renamed as *E. persicina* by Euzéby (1998).

A previously unreported leaf spot disease of the common bean (*Phaseolus vulgaris*) was observed in southeastern Spain (Almeria, Granada, and Malaga provinces) in November 2003 (González et al. 2005) and caused crop losses as high as 50 %. In 2004, samples of the pea (*Pisum sativum*) cultivar "Donna", with chlorotic and necrotic spots in leaves and tendrils caused by *E. persicina* (*Ep*), were collected from Granada and processed for microbiological analysis (González et al. 2007). Bacteria isolated from the symptomatic leaves were determined to be fermentative on the basis of their ability to metabolize glucose under aerobic and anaerobic conditions, and were finally identified as *Ep* by 16S rDNA (González et al. 2007).

In 2005, the *Ep* HKI 0380 strain was isolated from biofilms located on palaeolithic rock paintings in the "Cave of Bats" in Zuheros, southern Spain (Kiessling et al. 2005). It attracted attention due to the production of considerable quantities of slime (Kiessling et al. 2005).

In 2007, *Ep* was observed to cause necrosis for three new hosts of cucumber, tomato and melon grown under greenhouse conditions (Diánez et al. 2007). In March of 2008, three *Ep* strains were isolated from diseased seedling stems of pea (*P. sativum*) exported from Australia to China, which caused necrosis in the stem and tendril, and chlorosis in the leaf (Cui et al. 2009). In 2012, *Ep* was also isolated from apple and pear (Gehring and Geider 2012). That same year, three *Ep* strains (Cp1, Cp2 and Cp3) were isolated from lucerne (*Medicago sativa*) wilted sprouts in Gansu province, China, which caused sprout losses as high as 25 % (Zhang and Nan 2012).

Barak and Schroeder (2012) pointed out that bacterial food-borne pathogens use plants as vectors between animal hosts while following the life cycle script of plant-associated bacteria. As with other phytobacteria, *Ep* has a foothold in legume production areas. The commonality of environmental contamination translates to contact with plants (Barak and Schroeder 2012).

It remained unknown if *Ep* can survive in, and cause disease to, mature lucerne plants. The limits of host legume range, as well as the environmental adaptation capacity of *Ep*, also remained unknown. In addition, the specific mechanisms by which *Ep* might reach legume plants (e.g., lucerne) has not been determined.

With these factors in mind, the following two hypotheses concerning the *Ep* life cycle on lucerne were tested: (1) *Ep* might use one of three routes of contamination (i.e., water, soil and seed) to reach lucerne plants; or, (2) *Ep* spreads in two phases: from the infected plant to either soil or non-host plants, and then onto the uninfected plant. To reach a plant, *Ep* may travel from bulk soil to the rhizosphere or from the rhizosphere of one plant to another. Leaf epiphytes may be splash-dispersed from plant to plant or from the soil directly into the phyllosphere.

Given the scarcity of existing references on the pathogenesis of *Ep*, the objectives of experiments reported in the present paper were to: (1) validate the hypothesis of the life cycle of *Ep* on lucerne plants in germination trial and glasshouse experiments, respectively; (2) determine the host range of *Ep* in most forage or grain legumes found in Lanzhou City, China; and (3) study the environmental adaptation capacity of *Ep*. The ultimate aim of the present work was to illustrate that *Ep* may represent a new potential threat to forage or grain legumes within agricultural production systems.

Materials and methods

Seeds and *Ep* strain collection

Grain legume seeds (*Vigna angularis*, *Vigna unguiculata*, *Vigna radiata*, *Vicia faba*, *Phaseolus vulgaris*, *Dolichos lablab*, *Lens culinaris*, *Arachis hypogaea*, *Pisum sativum*, *Glycine max* and *Phaseolus lunatus*) were kindly supplied by the College of Agronomy, Gansu Agricultural University, Lanzhou. Forage legume seeds (*M. sativa*, *Onobrychis viciaefolia*, *Astragalus adsurgens*, *Trifolium repens*, *T. pretense*, *Vicia sativa*, *V. sepium*, *V. unijuga*, *Lespedeza bicolor*, *Lotus cornioulatus*, *Melilotus albus* and *M. officinalis*) were kindly supplied by the Lanzhou Official Herbage and Turfgrass Seed Testing Centre, Ministry of Agriculture, Lanzhou, China. *Ep* Cp2 strain (GenBank accession JN900058), was isolated from a wilted lucerne sprout (Zhang and Nan 2012) by the pasture crop phytopathology laboratory of the State Key Laboratory of Grassland Agro-Ecosystems, Lanzhou, China.

Ep inoculation suspension test

Thirty sixty-day-old pot (35 cm diameter and 25 cm in depth) lucerne plants were spray-inoculated with the Cp2 bacterial suspensions (10^6, 10^7, 10^8 and 10^9 colony forming units (CFU) per milliliter) as treatment and sterile distilled water (SDW) as control, six pots for each treatment and control. Thirty pots were kept within a plastic bag at 100 % humidity for 48 h, and then transferred to a greenhouse under natural conditions (day length 12–14 h, temperature 25–28 °C, and humidity 65–70 %). At 1, 3, 5, 7, 14 and 21 days after inoculation (DAI), inoculated leaflets were immersed in 75 % ethanol for 2 min, then in 1 % sodium hypochlorite for 5 min and subsequently rinsed five times in sterile distilled water (Nan 1995) to eliminate surface populations of bacteria. One 12-mm leaf disc was taken with a sterile cork borer from each leaflet and ground in 1 ml SDW and plated on maltose nutrient agar (MNA: maltose 5 g, peptone 10 g, beef extract 3 g, NaCl 5 g, agar 17 g, 1,000 ml water) plates after preparing a ten-fold dilution series. Samples (200 μl) of appropriate dilutions were spread onto MNA plates. The Petri plates were incubated at 28 °C overnight. Numbers of *Ep* colonies on each Petri plate were determined by counting single target colony. All single colonies of target bacteria having a fried-egg shaped colony, and producing a pink diffusible pigment on MNA (Zhang and Nan 2012).

Seed and water inoculation of *Ep* in germination trial

Unscarified lucerne seeds ("Algonquin") were used for the germination trials. Inocula were grown on nutrient agar (NA: peptone 10 g, beef extract 3 g, NaCl 5 g, agar 17 g, 1,000 ml water) for 24 h at 28 °C. Seeds were surface-sterilized in 75 % ethanol for 2 min, then in 1 % sodium hypochlorite for 5 min and subsequently rinsed five times in sterile distilled water (Nan 1995).

Surface-sterilized seeds were soaked in Cp2 bacterial suspensions of$\approx 1\times10^9$ CFU/ml for 10 min as seed inoculation treatments, and control seeds were steeped in sterilized tap water for 10 min. The inoculated or un-inoculated seeds were placed onto sterilized filter paper in glass Petri dishes (50 seeds per 9-cm dish) and moistened with 2 ml of sterile tap water.

Un-inoculated seeds on Petri dishes were periodically moistened with$\approx 1\times10^9$ CFU/ml bacterial suspensions of Cp2 as water inoculation treatments, and control seeds were moistened with sterile water. Three plates were used for each of the treatments and controls. The Petri dishes were placed in the incubator (Sanyo, MIR-524, Japan) at a constant 25 °C for 10 days. The entire experiment was repeated three times. The symptomatic tissues were sampled to re-isolate the *Ep* for fulfilling Koch's postulates.

Life cycle of *Ep* on the lucerne plant in a greenhouse experiment

Inocula were grown on NA for 24 h at 28 °C and lucerne seeds were surface-sterilized by the method of Nan (1995) for further research. Each pot containing sterilized soil innoculated with 10 ml Cp2 bacterial suspensions of$\approx 1\times10^9$ CFU/ml was sown with sterilized lucerne seeds as the soil inoculation treatment experiment. Controls were inoculated with sterilized tap water. These experiments were conducted within a greenhouse under natural conditions (day length 12–14 h, temperature 25–28 °C and humidity 65–70 %). Three replicate pots were used for each treatment and three seeds were sown in each pot. The growing seedlings were examined periodically and disease symptoms were recorded at 21 days after inoculation (DAI) with the pathogen (Sharma et al. 2005).

Sixty days after sowing, potted healthy lucerne plants were inoculated with 25 ml of the Cp2 spore suspensions ($\approx 1\times10^9$ CFU/ml) with an atomizer at 138 kPa or with sterilized water (controls). The inoculated pots were kept within a plastic bag at 100 % humidity for 48 h, and then transferred to a greenhouse under natural conditions. Three replicate pots were used for each treatment and three seeds were sown in each pot. Disease symptoms were observed and recorded at 14 DAI with the pathogen.

Forage or grain legumes as hosts of *Ep*, based on greenhouse evaluations

Pots containing sterilized soil were sown with healthy seeds of 11 grain legumes and 11 forage legumes for further studies. Inocula of the Cp2 strain were grown on NA for 24 h at 28 °C and then prepared as bacterial suspensions of$\approx 1\times10^9$ CFU/ml. Sixty-day-old grain or forage legume potted plants were spray-inoculated with the Cp2 bacterial suspensions as inoculation treatments. Controls were inoculated with sterilized water. This experiment was conducted within a greenhouse under natural conditions (day length 12–14 h, temperature 25–28 °C, and humidity 65–70 %). Three replicates were

used for each treatment, with three mature plants in each pot. The growing plants were examined periodically, the disease symptom and disease incidence being recorded at 14 days after inoculation with the pathogen. Severity of infection was evaluated on a 1-to-5 scale, in which 1 = no reaction and 5 = a severe response, as described by Thirthamallappa and Lohithaswa (2000). The symptomatic forage legume tissues were conducted to re-isolate the *Ep* for fulfilling the Koch's postulates.

Disease assessment

Disease incidence (percent wilting) was recorded 21 days after inoculation, according to the following formula:

$$\text{Disease incidence}\,\% = (\text{Number of wilted plants}/\text{Number of total plants}) \times 100$$

Bacterial isolation

At the end of the glasshouse experiments one gram of soil was removed from all pots and macerated in 100 ml sterile normal saline in a 150 rpm shaker for 30 min, then 200 μl suspensions from the top layer were plated onto MNA.

Samples from symptomatic stems, leaves, flowers, pods and seeds, after all glasshouse experiments had finished, were surface sterilized as described by Nan (1995), and dried on paper towels before excising 5 mm^2 subsamples taken from the margins of the symptomatic tissues. Subsamples were macerated in sterile porcelain spot plates using a sterile glass rod and approximately 1 ml of SDW. For colony differentiation, approximately 200 μl of the resulting macerates were plated onto MNA.

Plates were incubated (Sanyo incubator, MIR-524, Japan) at 28 °C for at least 48 h and then observed for colony formation. All single colonies of target bacteria having a fried-egg shaped colony, and producing a pink diffusible pigment (Zhang and Nan 2012) were subcultured directly onto MNA. All bacterial strains were re-isolated from potted soil and tissues of lucerne, then stored in 15 % glycerol at −70 °C for long-term storage, or cultured on NA and stored at 4 °C for short-term storage.

Target *Ep* strain identification

The colony characteristics of bacterial isolates on MNA plates were observed. The cell morphology of the bacterial isolates was examined under a microscope (Olympus BX51). Strand formation within the potassium hydroxide (KOH) test (Suslow et al. 1982) was used to identify presumptive gram-negative (GN, i.e., KOH positive) or gram-positive (GP, i.e., KOH negative) organisms. Target *Ep* confirmation was also made by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using *Ep* antiserum.

Environmental adaptation capacity of *Ep*

Cp2 strain inocula were prepared on NA for 24 h at 28 °C. One colony of Cp2 was transplated to nutrient broth (NB: peptone 10 g, beef extract 3 g, NaCl 5 g) for testing the growth curve of *Ep* over 96 h. Optimum growth temperature determinations for *Ep* was carried out at 4, 10, 25, 28, 35 and 40 °C, respectively. Optimum NaCl concentration for Cp2 was conducted in NB medium supplemented with 0 g/l, 5 g/l, 10 g/l, 20 g/l, 30 g/l and 40 g/l NaCl at the *Ep* optimum temperature. Optimum pH for Cp2 was studied in NB medium under pH concentrations of 3, 4, 5, 6, 7, 8, 9, 10 and 11 respectively (PHS-3C, China) at *Ep*'s optimum temperature and NaCl content. The relationship of *Ep* viability with moisture was carried out in NB medium supplemented within various PEG 6000-simulation drought conditions by the methods of Michel and Kaufmann (1973) at *Ep*'s optimum temperature, NaCl content and pH values. The relationship between *Ep* viability and light was investigated under three treatments of dark, light and alternating 12-h dark/light photoperiod conditions in NB medium at *Ep*'s optimum temperature, NaCl content and pH value. The OD600 values of environmental adaptation capacities (growth curve, temperature, moisture, pH, NaCl, light adaptation) of the growing Cp2 strain were measured, after 48 h growth, with a UV spectrophotometer (photoLab 6100, German).

Statistical analyses

Analysis of variance (ANOVA) was conducted for environmental adaptation capacities of *Ep*, including growth curve, temperature, pH, NaCl concentration and light adaptation, using SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Ep inoculation suspension test

The leaves of lucerne developed necrotic spots in 14 days after being inoculated with 10^9 concentration of *Ep* strain. However, the necrosis symptom of lucerne leaves was observed in 21 days after inoculation with 10^6, 10^7 and 10^8 concentrations of *Ep* strain. No symptom was detected in the control plants. The population of *Ep* increased with DAI. The growth of *Ep* at 10^9 concentration was significant higher than the other three concentrations (10^6, 10^7 and 10^8) (Fig. 1). The result showed that the 10^9 was the appropriate inoculation concentration for penetrating lucerne, which could be the prior inoculation concentration for pathogenicity identification.

Life cycle of *Ep* on lucerne

Seeds, irrigation and soil are the major routes for pathogen transmission in farming systems. To understand the possible ways *Ep* is transmitted, these three paths were tested. Both seed inoculation and water inoculation with *Ep* were shown (Fig. 2a) to cause lucerne sprouts to wilt within 10 days in the germination trial. Lucerne seedlings grown in soil inoculated with Cp2 bacterial suspensions were wilted (Fig. 2b). Sprayed *Ep* caused necrotic spots in leaves, stems, flowers and pods in the glasshouse experiments after 14 DAI. *Ep* was isolated from the soil after the glasshouse experiments were finished, demonstrating that *Ep* could survive in soil surrounding lucerne plant for >3 months. *Ep* seems to be a pathogen on the mature lucerne plant, causing necrosis in stems, leaves, flowers, pods and seeds by bacterial suspension spray inoculation (Fig. 2). All target *Ep* strains were re-isolated from used potting soil, necrotic stems, leaves, flowers, pods and seeds of lucerne. Identification was based on a negative Gram reaction, the fried-egg shaped colony formed, and a pink diffusible pigment produced on MNA plates, as well as a DAS-ELISA positive reaction.

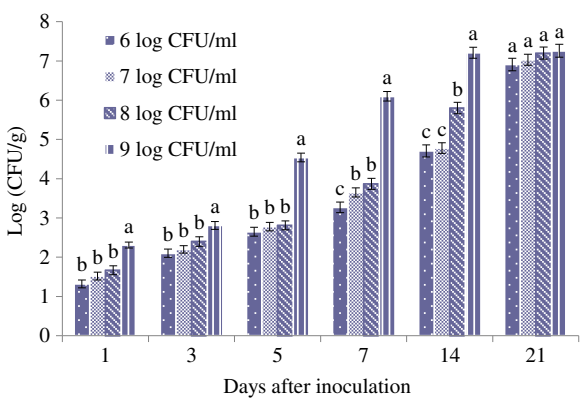

Fig. 1 Population of *Erwinia persicina* strain Cp2 in lucerne plant after different concerntration inoculation. Bars represent mean values (± SE) with differing letters denoting significant difference ($P<0.05$)

Host range of *Ep* on forage or grain legumes

Twenty two species within 15 genera of legume plants were evaluated in the study. *Ep* was determined to cause disease to 22 species of legume plants in the glasshouse experiments under natural conditions (Table 1). All specimens inoculated with *Ep* appeared to show similar symptoms. All the diseased legume plants from which *Ep* was isolated displayed symptoms such as stem chlorosis, leaf necrosis, pod necrosis and the appearance of necrotic spots on the leaves and flowers, irregular water-soaked spots on seeds, dropping of leaves, and conspicuous stem lesions as the whole plant wilted (Fig. 3). Likewise, symptoms shown by Brenner et al. (1994) and Gonzalez et al. (2005), to be caused by *Ep* in *Glycine max*, *Phaseolus vulgaris* and *Pisum sativum* were confirmed.

In addition, the pathogenic capacity of *Ep* was described for the first time in 19 species within 11 genera of legume plants in the present study glasshouse experiments. Twenty-two species of legume plants with these symptoms were positive in DAS-ELISA tests. Asymptomatic tissues and the negative controls were negative for *Ep* as indicated by DAS-ELISA. The disease incidence of forage or grain legumes after spray inoculation with *Ep* was 100 %, but the disease severity between the two types differed. The qualitatively worst disease was caused by *Ep* on the mung bean (*V. radiata*), and the least severity occurred with *O. viciaefolia*, *M. albus* and *M. officinalis*. Generally, the disease severity of grain legumes (mean value=2.545–3.727) caused by *Ep* was higher than for forage legumes (mean value=2–2.227) (Table 1).

Environmental adaptation capacity of *Ep*

The environmental adaptation capacity of *Ep*, including growth curve, optimum moisture content, temperature,

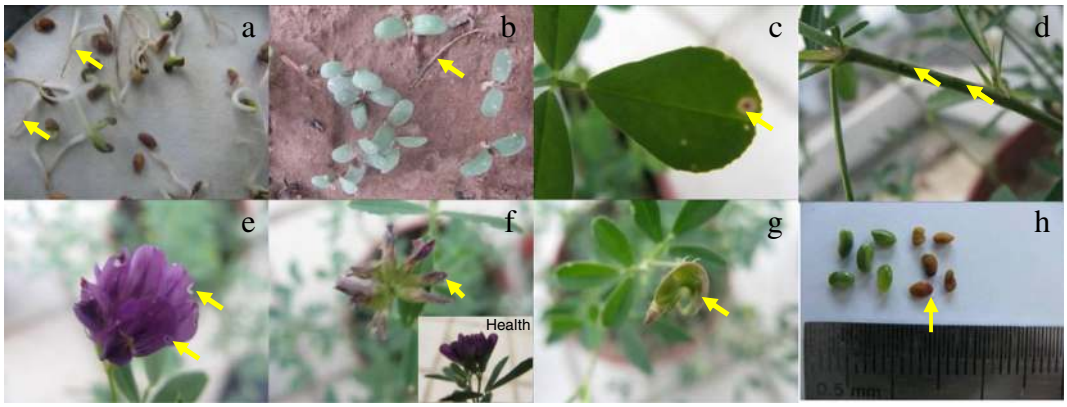

Fig. 2 Disease symptoms caused by *Erwinia persicina* observed on the lucerne plant. Wilting of seedlings caused by *E. persicina* when grown in petri dishes (**a**) and pots in the glasshouse (**b**); Necrotic spots on lucerne leaf, stem, flower caused by *E. persicina* in pot (**c**–**e**); Wilted flowers caused by *E. persicina* in pot (**f**); Necrotic spots on lucerne pod caused by *E. persicina* in pot (**g**); Lucerne seeds harvested from symptomatic pods that were wilted (**h**). Note: Arrows point to diseased tissues

pH, NaCl and light conditions were studied. The exponential phase of *Ep* was between 36 and 48 h (Fig. 4a). *Ep* grew best in 0 g/l PEG 6,000 and stopped growing in 500 g/l PEG 6,000 (Fig. 4b), with increasing concentrations progressively inhibiting growth, indicating a negative correlation to moisture content. The optimum

Table 1 Legume hosts susceptible to *Erwinia persicina* in the pot experiment

Scientific name	Disease parts of the host	Disease severity	New genus	New species
Vigna angularis	Seed, stem, leaf, flower, pod	3–4	X	X
Vigna unguiculata	Seed, stem, leaf, flower, pod	3–4		X
Vigna radiata	Seed, stem, leaf, flower, pod	3–5		X
Vicia faba	Seed, stem, leaf, flower, pod	3–4	X	X
Phaseolus vulgaris	Seed, stem, leaf, flower, pod	2–3		
Dolichos lablab	Seed, stem, leaf, flower, pod	3–4	X	X
Lens culinaris	Seed, stem, leaf, flower, pod	2–3	X	X
Pisum sativum	Seed, stem, leaf, flower, pod	2–3		
Arachis hypogaea	Seed, stem, leaf, flower, pod	3–4	X	X
Glycine max	Seed, stem, leaf, flower, pod	2–4		
Phaseolus lunatus	Seed, stem, leaf, flower, pod	2–3		X
Onobrychis viciaefolia	Seed, stem, leaf, flower, pod	2	X	X
Astragalus adsurgens	Seed, stem, leaf, flower, pod	2–4	X	X
Trifolium repens	Seed, stem, leaf, flower, pod	2–3	X	X
Trifolium pretense	Seed, stem, leaf, flower, pod	2–3		X
Vicia sativa	Seed, stem, leaf, flower, pod	2–3		X
Vicia sepium	Seed, stem, leaf, flower, pod	2–3		X
Vicia unijuga	Seed, stem, leaf, flower, pod	2–3		X
Lespedeza bicolor	Seed, stem, leaf, flower, pod	2–3	X	X
Lotus cornioulatus	Seed, stem, leaf, flower, pod	2–3	X	X
Melilotus albus	Seed, stem, leaf, flower, pod	2	X	X
Melilotus officinalis	Seed, stem, leaf, flower, pod	2		X

Symbols: "X" = first report as host genus and/or species, worldwide

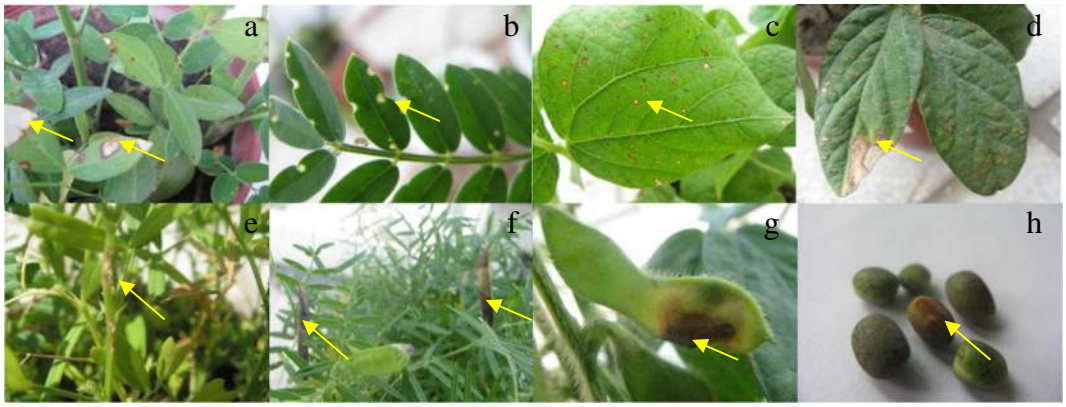

Fig. 3 Disease symptoms caused by *Erwinia persicina* observed on legumes for grain or forage. Leaf necrotic spots on leaves of *Astragalus adsurgens*, *A. adsurgens*, *Vigna angularis*, and *Glycine max*, respectively (**a**–**d**); Necrotic spots on stems of *Vicia sepium* and *V. sepium*, respectively (**e** and **f**); Necrotic spots on pods of *G. max* (**g**); Water-soaked symptom on seed of *V. sativa* (**h**). Note: Arrows point to diseased tissues

temperature for Ep was 28 °C, within a tolerated range of 10–35 °C (Fig. 4c). It also grew slowly within the 5–10 °C or 35–40 °C ranges, but ceased growth below 5 °C or above 40 °C. *Ep* is adapted to a wide pH range (4–11), but the optimum pH for *Ep* growth was 7.0 (Fig. 4d). Although *Ep* grew well under the zero sodium chloride condition, 0.5 % was significantly better. *Ep* endured the 3 % NaCl concentration, but would not grow at 4 % (Fig. 4e). The best light treatment condition for *Ep* growth was the 12 h light/dark photoperiod condition. Bacterial growth occurred under all light conditions however, it was most significant under a 12 h light/dark photoperiod (Fig. 4f).

Discussion

The life cycle of *Ep* on lucerne was investigated by glasshouse experiments under controlled conditions. *Ep* could be transmitted by seeds, water, and soil, as with the human pathogens *Escherichia coli* and *Salmanella enterica* (Watanabe et al. 1999; CDC 2012). The results of soaking-seed, infesting-soil and spray-inoculation with *Ep* suspensions had shown that *Ep* could invade tested lucerne through natural openings. Once on a plant, *Ep* may use flagella-mediated motility to traverse the plant spheres: rhizosphere (spermosphere) to phyllosphere, and then onwards to the inflorescences, and subsequently the seeds. As with other quarantine pathogens such as, *Clavibacter michiganensis* subsp. *michiganensis* (Davis et al. 1984; De León et al. 2011) and *C. m.* subsp. *insidiosus* (OEPP/EPPO 2010), *Ep* was found to be a seedborne pathogen. Bacteria present on seeds can be internalized within the sprout and become protected from post-harvest sanitation (Itoh et al. 1998; Warriner et al. 2003). Internally, seed-borne bacteria may remain alive indefinitely (Neergaard 1977). Bacteria movement over long distances is facilitated by dispersed seeds, which explains its distribution throughout all of the lucerne-growing regions of the world. Practically speaking, seed transmission is the most likely way for bacteria to be introduced to previously disease-free areas (Nemeth et al. 1991).

Most legume species in the world with the exception of Glycine max (Brenner et al. 1994), Phaseolus vulgaris (González et al. 2005), Pisum sativum (González et al. 2007) and Medicago sativa (Zhang and Nan 2012) have not been previously tested with *Ep*. These leguminous species may aid in the over-wintering of the bacteria and provide a source of inocula at the beginning of the growing season. In the present study, 22 species within 15 genera were chosen to determine the pathogenicity of *Ep*, and 19 species within 11 genera of legume plants in the glasshouse experiments were found to be potential new hosts. Brenner et al. (2005) pointed out that *Erwinia* species cause plant diseases that include mainly blights and wilts, and the pathogen was described as usually starting its damage to vascular tissue by entry through natural openings and wounds, and subsequently spreading throughout the plant. The present study, using the spray inoculation method, demonstrated that *Ep* could invade tested forage or grain legumes in such a manner, and ultimately be

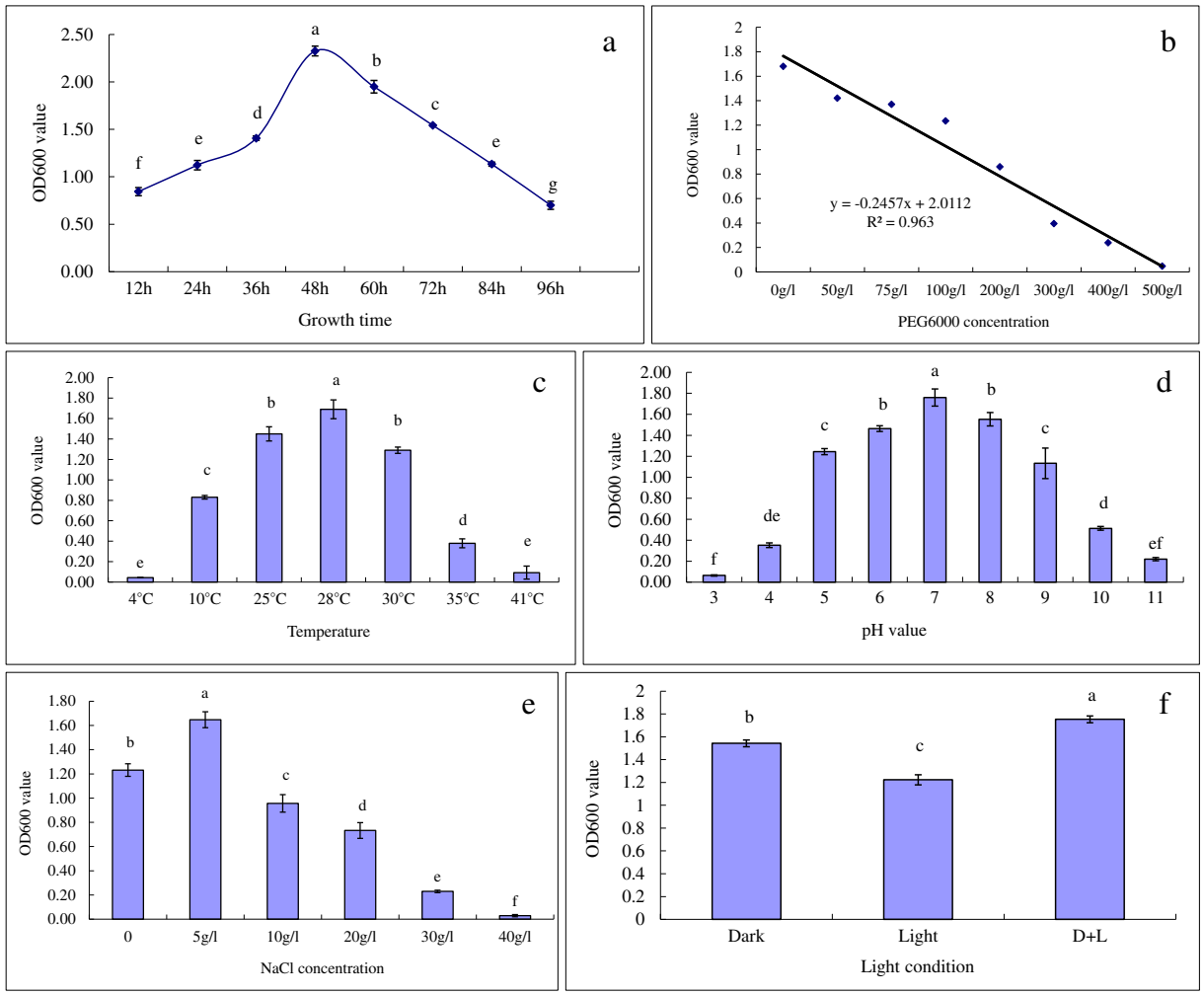

Fig. 4 The environmental adaptation capacities of *E. persicina*, including growth curve (**a**), moisture (**b**), temperature (**c**), pH (**d**), NaCl (**e**), and light adaptation (**f**). Bars represent mean values (± SE) with differing letters denoting significant difference ($P<0.05$). "D+L" represents the 12 h dark/light photoperiod condition

transmitted to the seed. Knowledge of the host range will help to predict potential over-wintering sites and the level of primary inocula from these sites that might be available to infect legume fields at the start of the growing season. It will also help define species and prioritize areas to be examined. Researching the newly defined host species will result in a greater understanding of their natural infection as well as increase knowledge of their importance in the over-wintering of *Ep*.

The optimum temperature of *Ep* was in accordance with the results of Hao et al. (1990). As with most microbes, *Ep* growth declined with moisture content in the natural environment, but *Ep* could also grow under a lower water potential. In addition, *Ep* was not sensitive to light. The wide range of pH values and NaCl concentrations compatible with *Ep* growth has indicated that it could survive within a wide range of environmental conditions, including various farming lands and greenhouses. Furthermore, *Ep* could endure harsh growth conditions like arid, saline and alkaline environments, remaining quiescent until presented with an opportunity for germinating within the appropriate conditions, but subsequently bursting into exponential growth within 48 h.

In conclusion, *Ep* is a newly characterized pathogen which can complete its whole life cycle on legume plants and is conserved in soil and plant seeds. Kiessling et al. (2005) reported that *Ep* produced large quantities

of extracellular polysaccharide (EPS) and a number of signalling substances like acylated homoserine lactones. EPS constitutes an appropriate matrix for embedding diverse microorganisms in the course of biofilm formation (Kiessling et al. 2005). Biofilms are ubiquitous in nature, and the majority of human bacterial infections involve biofilms (Davies 2003; Hall-Stoodley et al. 2004). While biofilms contain cells with a heterogeneous range of states (Kim et al. 2009), on average, bacteria in biofilms have a much higher (up to 1,000-fold) antibiotic tolerance than their planktonic counterparts (Ceri et al. 1999). Therefore, it is possible that *Ep* may not be easily eliminated because of the protection afforded by this biofilm or the seed coat. Once *Ep* enters into a new region, it may represent a new and permanent threat to forage or grain legumes production within its farming systems.

Acknowledgements We would like to thank Prof. Yanrong Wang for supplying the forage legume seeds, Prof. Xiurong Chen and Dr. Xingxu Zhang for technical assistance. We are grateful to Ross Booth and two anonymous reviewers for reviewing the manuscript. This research was financially supported by the National Key Basic Research Program (973) of China (2014CB138704) and National Construction of Modern Agricultural Industrial Technology Systems of China – Forage Industrial Technology Systems.

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