Fast recognition of *Lecanicillium* spp. and its virulence against *Frankliniella occidentalis*

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Abstract

Background: *Frankliniella occidentalis* (Thysanoptera: Thripidae) is a highly destructive sucking pest of numerous crops. The entomogenous fungi of *Lecanicillium* spp. are important pathogens of insect pests, and some have been developed as commercial biopesticides. In order to explore the wild resources of *Lecanicillium* spp. in the development of more effective *F. occidentalis* controls, efficient gene combinations for strain identification were screened from internal transcribed spacer (ITS) and the genes *SSU*, *LSU*, *TEF*, *RPB1*, and *RPB2*.

Results: In this study, six genes were used to reconstruct the phylogeny of *Lecanicillium* genus respectively. Among these, only four events (ITS, *TEF*, *RPB1*, *RPB2*) were validated using the gold standard for identifying the strains. All the phylogenetic trees reconstruct by free combination of these four events exhibited the same topology. Bioassay studies of a purified conidial suspension further confirmed the infection of second-instar nymphs and adult female *F. occidentalis* by seven strains of *Lecanicillium*. *L. attenuatum* strains GZUIFR-lun1405 was the most virulent, approximately 8.33% of adult and 23.33% of nymphs *F. occidentalis* survived after a 7-d exposure. Two strains were selected to compare the effects on the amount of eggs laid by *F. occidentalis*. The number of nymphs of *F. occidentalis* significantly decreased when adult *F. occidentalis* were treated with *L. cauligalbarum* strain GZUIFR-ZHJ01 compared to the controls.

Conclusions: We found that combination of ITS and *TEF* could be used for fast recognition of *Lecanicillium* spp. We demonstrated that *L. attenuatum*, *L. cauligalbarum*, *L araneogenum*, and *L. aphanocladii* had various efficacies against *F. occidentalis* which were the first to demonstrate efficient control of *F. occidentalis*. In addition, the *L. cauligalbarum* strain GZUIFR-ZHJ01 not only caused high mortality in *F. occidentalis* but also inhibited the fecundity of the pest.

Backgroud

Frankliniella occidentalis (Pergande) is a highly polyphagous herbivore that is an economically pernicious pest of many crops and results in especially serious losses to the vegetable and flower industries [1, 2]. It feeds on the tender parts of crops and flowers or oviposits within the plant tissue, reducing the productivity and quality of the crop. When it feeds on the contents of individual cells, it causes a reduction in photosynthetic capacity [3]. The pest also facilitates the transmission of plant viruses, such as tomato spotted-wilt virus and impatiens necrotic-spot virus [4, 5]. *F. occidentalis* was first described in California, USA; although it received little attention at the time, it subsequently became a major international pest facilitated by increasing international agriculture exchanges, with the first pesticide-resistant strain discovered in the 1970s [6-8]. *F. occidentalis* was first reported as an invasive pest in Beijing in 2003 [9]. It then gradually spread to many provinces in China, where it has been a dominant pest of floriculture and vegetable crops. [10]. At present, it can be found in more than 10 provinces throughout China [11]. Additionally, *F. occidentalis* has been predicted to spread to and successful overwinter in more northern areas of China because of climate change [12].

Presently, the most common control measures are chemical pesticides, but their efficacy is often poor because of the habit of the pest to reside within the flowers and buds, where it is difficult to reach with the spray [7, 13, 14]. Even more serious are the varying degrees of resistance that are developing due to the overuse of pesticides [15-17]. As a consequence, biological control has been receiving an increasing amount of attention. The representative entomopathogenic fungi of *F. occidentalis,* such as *Beauveria bassiana* [18-20], *Metarhizium anisopliae* [21-23], *Lecanicillium lecanii* [24-26], and *Isaria fumosorosea* [27, 28], are of particular interest to researchers [29]. Unfortunately, very little is known about species' ability to control *F. occidentalis*, other than these typical representative species. Therefore, finding new entomopathogenic fungi capable of controlling *F. occidentalis* is a priority for our research group because of the needs and demands of the majority of crop growers [29].

As insect pathogens, *Lecanicillium* spp. are effective biological control agents for numerous diseases, insect pests, and plant-parasitic nematodes. [30]. So far, 15 commercial preparations based on *Lecanicillium* spp. have been or are currently being developed [31]. Kepler et al. (2017) concluded that *Lecanicillium* should be incorporated into *Akanthomyces* and formally transferred a number of *Lecanicillium* species. However, the compatibility of *Lecanicillium* was not so good. In addition, in the paper published later, scholars also used the name *Lecanicillium*, for example *Lecanicillium testudineum* and *Lecanicillium restrictum* [32], *Lecanicillium subprimulinum* [33], *Lecanicillium coprophilum* [34]. In order to better use *Lecanicillium* to control *F. occidentalis*, we will continue to use the previous taxon to carry out our work.

The *Lecanicillium* genus is typified by *L. lecanii*, with *Torrubiella confragosa* as the sexual morph [35, 36]. *L. lecanii* was first named *Cephalosporium lecanii* Zimm. by Zimmermann in 1898. Viegas subsequently incorporated the species into *Verticillium* Nees in 1939 [37]. More recently, Zare and Gams (2001) redefined the genus, placing all the insect pathogens into a newly established genus, *Lecanicillium*, using the morphological and sequence data for the internal transcribed spacer (ITS) rDNA. Presently, 30 *Lecanicillium* species have been formally described and are listed in the Fungorum Index (http://www.indexfungorum.org). Considering that the information provided by ITS was limited, several researchers have used different genes to identify new *Lecanicillium* species, including two new *Lecanicillium* species (*L. araneogenum* and *L. uredinophilum*), which were identified based on multilocus (*TEF*, *RPB1*, *RPB2*, *LSU,* and *SSU*) sequence data [38, 39]. Two other new species (*L. sabanense* and *L. cauligalbarum*) were identified based on phylogenetic analysis of combined multilocus and ITS sequences [40, 41]. However, it would be laborious if six genes were required to identify each newly collected strain. Some scholars suggest that ITS regions and at least one protein gene with sufficient genetic variation, such as *TEF* or *RPB2*, should be sufficient, rather than using the rather conserved 28S or 18S rDNA [42]. Although, it is unclear whether this advice is appropriate for *Lecanicillium* spp; therefore, screening for the most efficient possible gene combinations for identifying the fungal strains will have particular significance for the application of *Lecanicillium* spp. in the control of *F. occidentalis*. These data will facilitate the exploitation of wild resources of *Lecanicillium* spp. and remove the current limitations preventing the exploitation of new strains for biological control, allowing potential fungal pathogens to be screened from species other than the well-understood preserved strains.

Methods

Entomopathogenic fungi

The specimens were collected from forest or tea plantations from 2014 to 2019 in Guizhou, China. These specimens were preliminarily identified as *Lecanicillium* spp. by their morphology and some were identified, while others were not. All the strains were deposited in GZAC, Guizhou University, Guiyang. The details of the strains are listed in Table 1.

Insects

F. occidentalis were collected from vegetable plants in the Guiyang area of Guizhou Province, China, and were used to establish a laboratory colony. The individuals were reared and maintained on kidney bean pods in the laboratory of the Institute of Entomology, Guizhou University for 12 years (2006 to 2018). Thrips were reared in an artificial-climate chest, with a photoperiod of 14h L:10h D, temperature of 25 ± 1℃, and relative humidity of 70 ± 5%. Adult females and second-instar nymphs of approximately the same age were selected as sources for the experiment.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted using a previously described method. The primers used for PCR amplification of the ITS region, *SSU*, *LSU*, *TEF*, *RPB1*, and *RPB2* followed those used in the references [41].

Comparison of combination identification

The DNA sequences used in this study were edited using the LASERGENE software (version 6.0, DNASTAR). The sequences downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) comprised 29 *Lecanicillium* taxa and one sequence from *Simplicillium lanosoniveum*, which was used as the outgroup [33]. A total of 46 sequence submissions were selected and are listed in Table 2. Multiple sequence alignments for *TEF*, *RPB1*, and *RPB2* were performed in MAFFT with the default settings [43]. Multiple sequence alignments for ITS, *LSU*, and *SSU* were conducted using MUSCLE algorithm [44] from MEGA 6 [45]. The sequences were edited manually. A multiple alignment of the combined partial sequences was assembled with MEGA 6 [45]and SEQUENCEMATRIX 1.7.8 [46]. The command 'hompart' in PAUP* 4.0b10 was used for assessment of concordance amongst the genes and the ITS region [47].

Bayesian inference (BI) was performed using MRBAYES 3.2 [48] and maximum likelihood (ML) analysis was performed using RAxML [49] to analyze the data. The ML and BI analyses were performed on each of the six genes (ITS, *LSU*, *SSU*, *TEF*, *RPB1*, *RPB2*). Portfolio analysis was performed the ML and BI analyses according the consequence of the single gene analysis. Nucleotide substitution models were determined by MrModeltest 2.3 [50]. For BI, 10 000 000 generations were performed, with one tree selected every 500th generation. For ML, the model GTRGAMMA was used, and a bootstrap analysis with 1000 replicates was performed to assess statistical support for the tree topology. Phylogenetic trees were viewed with TREEGRAPH.

Bioassay

First, the virulence of seven *Lecanicillium* spp. strains to second-instar nymphs and adult females was tested. Second, the biometrics of the adults and the number of nymphs hatched under each strain treatment were compared. Strains that significantly reduced the number of nymphs were subjected to a preliminary exploration of the reduced thrips fecundity to verify whether the strain directly affected fertility and hatchability.

Preparation of spore suspension

The strains were routinely grown on potato dextrose agar. Plates were incubated at 25°C for 14 days and aerial conidia were harvested by flooding the plate with 0.025% Tween-80. The conidial suspension was vortexed for 1 min and filtered through double layers of lens paper. The final spore concentrations were resuspended to 2×10^8 conidia ml^{-1} using a hemocytometer.

Inoculation of the adult females

The females were inoculated using the leaf dipping method with some modifications (bean pods were used instead of leaves): The bean pods were cut into long strips of equal size and cut longitudinally. They were placed in the prepared spore suspension for 15 s, then removed and allowed to dry naturally. The pods were fixed with a paper clip placed in the fingertip tube. Twenty female adult (2–3 days old) *F. occidentalis* were transferred with suction trap into the fingertip tube and sealed in with cotton wool. The experiment was repeated four times, and 0.025% twain-80 solution was used as the control. The pods were placed in a light incubator with a temperature of 25 ± 1°C, relative humidity of 70%, and photoperiod L:D of 12:12 h, and we observed the thrips every day for 7 consecutive days and recorded the number of deaths. At the end of the experiment, dead adults were removed and placed in a petri dish with wet filter paper at the bottom for wet culture, and those with that showed spore structure after incubation were considered as effectively infected.

Inoculation of the second-instar nymphs

The leaf dipping method was adapted by cutting the leaves of common beans into small round blocks of about 1 cm^2. The leaves of the common bean were soaked in the prepared spore suspension for 15 s and then removed and dried naturally. Thirty second-instar nymphs were put into a 25-mL conjoined plastic dip box (4.3 × 3.1 × 3 cm, with the mesh glued to the holes at the top). The experiment was repeated four times, and 0.025% twain-80 solution was used as the control. The nymphs were placed into a light incubator with a temperature of 25 ± 1°C, relative humidity of 70%, and a photoperiod L:D of 12:12 h, and we observed the thrips every day for 7 consecutive days and recorded the number of deaths. At the end of the experiment, dead adults were removed and placed in the petri dish with wet filter paper at the bottom for wet culture, and those that showed spore structure after incubation were considered as effectively infected.

Effects on the fertility and hatchability of F. occidentalis

Given that eggs of thrips are laid inside the bean pod tissue and are not easily visualized, the amount of eggs could not be determined. The difference in the hatchability was determined by comparing the number of nymphs that appeared after the same number of thrips had laid their eggs over the same period of time. There were two experiments, the first involved treating the bean pods within which thrips had deposited eggs, and the other involved treating bean pods without eggs. The difference caused by the treatment after eggs were laid represented the difference in hatchability, while the difference caused by the treatment before eggs were laid represented the difference caused by both fertility and hatchability.

In the first experiment, 20 female adult (2–3 days old) *F. occidentalis* were transferred with suction trap into the fingertip tube and sealed with cotton wool. After 24 h, the pods were placed into the spore suspension for 15 s and transferred into the new fingertip tube to identify the effects on the hatchability of eggs in pods. In the second experiment, pods were placed into the spore suspension for 15 s and transferred into the new fingertip tube. Twenty female adults (2–3 days old) of *F. occidentalis* were transferred with suction trap into the fingertip tube and immediately sealed in with cotton wool. Each experiment was repeated nine times, and 0.025% twain-80 solution was used as the control. The pods were placed in a light incubator with a temperature of 25 ± 1°C, relative humidity of 70%, and photoperiod L:D of 12:12 h, and we recorded the number of nymphs that emerged after 7 days.

Statistical analyses

The mortality of *F. occidentalis* was analyzed using an ANOVA one-way model with SPSS software (version 18.0; SPSS, Chicago, IL, USA). Tukey's honestly significant difference test ($P < 0.05$) was used to analyze the survival rate of the adults and nymphs and number of nymphs that hatched. Inverse sine square root transformation was used for the percentage data before the ANOVA performance. All the data were represented as mean ± SE. The corrected percent mortality was calculated by the Abbott's formula [51].

Results

Sequencing and phylogenetic analysis

Two classic phylogenetic tree building software programs (MRBAYES 3.2 and RAxML) were used to identify seven strains (Table 1) using six genes (ITS, *SSU*, *LSU*, *TEF*, *RPB1*, *RPB2*) respectively. Among these, only four events (ITS, *TEF*, *RPB1*, *RPB2*) were validated using the gold standard for identifying the strains (phylogenetic incongruence with statistical measures of confidence; Fig. S1–S4). No significant differences in topology were observed between the BI and ML phylogenies. The phylogenetic relationship with *LSU* or *SSU* was not well supported. The first tree formed with almost all the *Lecanicillium* species (only *L. evansii* could not be found in the NCBI) and one *Simplicillium* species (*S. lanosoniveum*). The sequence dataset consisted of 2735 bases, including inserted gaps (ITS: 523 bp; *TEF*: 772 bp; *RPB1*: 555 bp; *RPB2*: 885 bp). GZUIFR-lun1404 and GZUIFR-lun1405 formed an independent branch in a polytomy

together with a clade containing *L. attenuatum* (BI posterior probabilities 0.99, ML bootstrap 98%). GZUIFR-lun1403 formed an independent branch in a polytomy together with a clade containing *L. lecanii* (BI posterior probabilities 0.98, ML bootstrap 97%). Strain GZU1032Lea and *L. araneogenum*, strain GZUIFR-lun1505 and *L. aphanocladii*, strain GZUIFR-ZHJ01 and *L. cauligalbarum*, and strain GZUIFR-huhu and *L. tenuipes* formed independent branches (BI posterior probabilities 1, ML bootstrap 100%) (Fig. 1). The other phylogenetic trees exhibited the same topology (Fig. S5–S9 and Fig. 2).

Comparison of the virulence of the seven fungal strains infecting F. occidentalis

The results show that the strain GZUIFR-huhu was non-toxic to *F. occidentalis*. All the other six strains were pathogenic to *F. occidentalis*, but their virulence varied (Fig. 3). *L. attenuatum* strains GZUIFR-lun1404 and GZUIFR-lun1405 were the most virulent of the six strains from the five species. Approximately 8.33% of adult *F. occidentalis* survived after a 7-d exposure to these two *L. attenuatum* strains.

Five strains showing effectiveness against *F. occidentalis* adult females were also pathogenic to second-instar nymphs. *L. attenuatum* strain GZUIFR-lun1405 was the most virulent, with approximately 23.33% nymphs surviving after a 7-d exposure (Fig. 4).

Differences were found in the number of nymphs hatched by female *F. occidentalis* after exposure to different strains. There were no significant differences among strain GZUIFR-huhu, *L. attenuatum* strain GZUIFR-lun1405, and control groups. The group treated with *L. cauligalbarum* strain GZUIFR-ZHJ01 had the fewest survivors, significantly fewer than in the other treatment and control groups (Fig. 5).

L. attenuatum strain GZUIFR-lun1405 and *L. cauligalbarum* strain GZUIFR-ZHJ01 were selected to compare the effects on the amount of eggs laid by *F. occidentalis*. There was no significant difference between the experimental and control groups for the number of nymphs hatched from the same number of eggs (Fig. 6). However, the number of nymphs of *F. occidentalis* significantly decreased when adult *F. occidentalis* were treated with *L. cauligalbarum* strain GZUIFR-ZHJ01 compared to the controls (Fig. 6). Clearly, the *L. cauligalbarum* strain GZUIFR-ZHJ01 effected the number of eggs laid by *F. occidentalis*.

Discussion

With the development of molecular methods, an increasing number of biodiversity researchers find it easier to identify species by DNA barcoding than by morphological methods [52, 53]. The success of DNA barcoding has facilitated the characterization of bacterial biodiversity in every nook and cranny on the planet [54, 55]. Compared to bacteria, fungi are very difficult to definitively identify because they contain large amounts of non-coding and repetitive DNA (Mohanta & Bae 2015). Internal Transcribed Spacer (ITS) sequences have been shown to be useful in delineating many fungal species (Irinyi et al. 2015, Vu et al. 2016); unfortunately, few fungal species' ITS sequences are available in GenBank (Hawksworth & Lücking 2017), and many of the sequences are of poor quality (Nilsson et al. 2006, Vu et al. 2016). Therefore, multilocus (*TEF*, *RPB1*, *RPB2*, *LSU*, and *SSU*) sequence data are used to identify the

phylogenetic relationship of some fungi, such as the Ophiocordycipitaceae [56, 57]. *Lecanicillium* species form part of Ophiocordycipitaceae and are also best identified by these multilocus sequences [41]. In the present study, *LSU* and *SSU* could not successfully identify all species of *Lecanicillium*; however, *TEF*, *RPB1*, and *RPB2* were found to be useful for identifying all species. Any one or more of these three genes combined with ITS could also clearly distinguish the *Lecanicillium* genus. We believe a combination of ITS and *TEF* is optimal for the efficient recognition of *Lecanicillium* spp., according to the richness of each sequence.

The two phylogenetic trees created in this study formed with almost all the *Lecanicillium* species (only *L. evansii* could not be found in the NCBI). The species identified in the previous papers aggregated with their corresponding sequences and maintained high approval ratings (Table 1). Therefore, the strain GZUIFR-lun1403 was identified as *L. lecanii.* Although the strain GZUIFR-huhu was close to *L. tenuipes* in the phylogenetic tree, the frequency of base substitutions indicated a clear distinction from *L. tenuipes*; therefore, we considered it to be a new species.

At present, the microbial agents used for *F. occidentalis* control mainly belong to *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea*, and *Lecanicillium lecanii*, etc [29]. We found that *L. attenuatum*, *L. cauligalbarum*, *L. araneogenum*, and *L. aphanocladii* were pathogenic to *F. occidentalis*, but their virulence varied. This is the first report on the pathogenicity of the strains to *F. occidentalis*.

In addition to the direct pathogenicity, sublethal effects of fungus on thrips, including fecundity, longevity, and feeding ability, may be one of the most important aspects of exposure to microbial control agents [29]. Zhang et al. (2015) reported that a strain of *B. bassiana,* GZGY-1-3, significantly reduced the reproductive success of *F. occidentalis* and the subsequent fitness of their progeny. Additionally, an *M. anisopliae* strain significantly reduced the fecundity, egg fertility, and longevity of *Megalurothrips sjostedti* adults [58]. In this article, the *L. cauligalbarum* strain GZUIFR-ZHJ01 did not result in the highest mortality rate of adults or nympha, but it significantly reduced the number of eggs lain by *F. occidentalis*. This effect could be an important factor to measure in the development of future *F. occidentalis* control projects. It is possible that the fungus competes with the host for nutrients and, consequentially, physiologically interferes with egg fertility. Therefore, this sublethal effect could partly offset the relatively slow death rate of fungal infection compared to conventional insecticides and may play an important role in thrips control [29]. Further study is needed to determine whether, like other entomogenous fungi, the *L. cauligalbarum* strain GZUIFR-ZHJ01 has other sublethal effects on *F. occidentalis*.

Conclusions

Our study demonstrated that combination of ITS and *TEF* could be used for fast recognition of *Lecanicillium* spp. We demonstrated that *L. attenuatum*, *L. cauligalbarum*, *L araneogenum*, and *L. aphanocladii* had various efficacies against *F. occidentalis* which were the first to demonstrate efficient control of *F. occidentalis*. In addition, the *L. cauligalbarum* strain GZUIFR-ZHJ01 not only caused high mortality in *F. occidentalis* but also inhibited the fecundity of the pest.

Abbreviations

Spp.: Species pluralis; GZAC: the Institute of Fungal Resources of Guizhou University; rDNA: ribosomal DNA; LSU: the large subunits of the rDNA; SSU: the small subunits of the rDNA; TEF: the transcription elongation factor-1α; ITS: ITS1–5.8S rDNA–ITS2 region, the first and the internal transcribed spacers; RPB1: DNA-directed RNA polymerase II subunit rpb1; RPB2: DNA-directed RNA polymerase II subunit rpb2.

Declarations

Ethics approval and consent to participate

Frankliniella occidentalis samples were maintained at our greenhouse at Institute of Entomology, Guizhou University, China- no permission required.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

Y.M.Z. performed experiments, analysed data and wrote the article. X.Z. provided strains of fungus and revised the manuscript. J.R.Z. designed experiments and revised the manuscript. T. J. analysed data. J.Q.X. performed experiments. All the authors reviewed the manuscript.

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Tables

Table 1 Specimen information and the host information of the species in this study

Voucher Information	Species	Host	Refrence
GZUIFR-huhu	*Lecanicillium* sp.	spider	This work
GZUIFR-lun1403	*Lecanicillium* sp.	soil	This work
GZUIFR-lun1404	*Lecanicillium attenuatum*	*Empoasca flavescens*	(Zhou et al. 2016)
GZUIFR-lun1405	*Lecanicillium attenuatum*	*Empoasca flavescens*	(Zhou et al. 2016)
GZUIFR-lun1505	*Lecanicillium aphanocladii*	soil	(Zhou et al. 2018b)
GZUIFR-ZHJ01	*Lecanicillium cauligalbarum*	stemborer	(Zhou et al. 2018a)
GZU1032Lea	*Lecanicillium araneogenum*	spider	(Chen et al. 2017)

Table 2 Specimen information and GenBank accession numbers used in this study

Species Voucher Information	*LSU*	*SSU*	*TEF*	*RPB1*	*RPB2*	*ITS*
GZUIFR-huhu	**MN944419**	**MN963916**	**MT006068**	**MT006058**	**MT006063**	**MN944445**
GZUIFR-lun1403	**MN944420**	**MN963917**	**MT006069**	**MT006059**	**MT006064**	**MN944446**
GZUIFR-lun1404	**MN944421**	**MN963918**	**MT006070**	**MT006060**	**MT006065**	**KT345700**
GZUIFR-lun1405	**MN944422**	**MN963919**	**MT006071**	**MT006061**	**MT006066**	**MN944447**
GZUIFR-lun1505	**MN944423**	**MN963920**	**MT006072**	**MT006062**	**MT006067**	**MN944448**
GZUIFR-ZHJ01	MH730663	MH730665	MH730667	MH801920	MH801922	MH801924
GZU1032Lea	KX845704	KX845706	KX845698	KX845670	KX845702	
Lecanicillium acerosum CBS418.81	KM283786	KM283762	KM283810	KM283832	KM283852	EF641893
L. antillanum CBS350.85	AF339536	AF339585	DQ522350	DQ522396	DQ522450	AJ292392
L. aphanocladii CBS797.84	KM283787	KM283763	KM283811	KM283833	KM283853	
L. aranearum CBS726.73a	AF339537	AF339586	EF468781	EF468887	EF468934	AJ292464
L. araneicola BTCC-F35						AB378506
L. araneogenum GZU1031Lea	KX845703	KX845705	KX845697	KX845699	KX845701	
L. attenuatum CBS402.78	AF339565	AF339614	EF468782	EF468888	EF468935	AJ292434
L. attenuatum KACC42493	KM283780	KM283756	KM283804	KM283826	KM283846	
L. bristletailum GZUIFRZHJ02	MH730664	MH730666	MH730668	MH801921	MH801923	MH801925
L. dimorphum CBS345.37	KM283788	KM283764	KM283812	KM283834	KM283854	
L. flavidum CBS300.70D	KM283789	KM283765	KM283813		KM283855	EF641877
L. fungicola var. aleophilum CBS357.80	KM283791	KM283767	KM283815	KM283835	KM283856	NR_111064
L. fungicola var. fungicola CBS992.69	KM283792	KM283768	KM283816		KM283857	NR_119653
L. fusisporum CBS164.70	KM283793	KM283769	KM283817	KM283836	KM283858	AJ292428
L. kalimantanense BTCC-F23						AB360356
L. lecanii CBS101247	KM283794	KM283770	DQ522359	KM283837	KM283859	JN049836
L. lecanii CBS102067	KM283795	KM283771	KM283818	KM283838	KM283860	
L. longisporum CBS102072	KM283796	KM283772	KM283819	KM283839	KM283861	
L. longisporum CBS126.27	KM283797	KM283773	KM283820	KM283840	KM283862	
L. muscarium CBS143.62	KM283798	KM283774	KM283821	KM283841	KM283863	
L. nodulosum IMI 338014R		EF513075				EF513012
L. pissodis CBS118231	KM283799	KM283775	KM283822	KM283842	KM283864	
L. primulinum JCM 18525	AB712263					AB712266
L. primulinum JCM 18526	AB712264					AB712267
L. psalliotae CBS532.81	AF339560	AF339609	EF469067	EF469096	EF469112	JN049846
L. psalliotae CBS101270	EF469081	EF469128	EF469066	EF469095	EF469113	
L. psalliotae CBS363.86	AF339559	AF339608	EF468784	EF468890		
L. restrictum CCF5252			LT626943			LT548279
L. sabanense JCHA5	KC875225	KC633251	KC633266		KC633249	KC633232
L. saksenae IMI 179841						AJ292432
L. subprimulinum HKAS99548	MG585315	MG585316	MG585317			MG585314
L. subprimulinum HKAS99549	MG585319	MG585320	MG585321			MG585318
L. testudineum UBOCC-A112180			LT992868			LT992874
L. testudineum UBOCC-A116026			LT992867			LT992871
L. tenuipes CBS309.85	KM283802	KM283778	DQ522341	KM283844	KM283866	JN036556
L. uredinophilum KACC44082	KM283782	KM283758	KM283806	KM283828	KM283848	
L. uredinophilum KACC47756	KM283783	KM283759	KM283807	KM283829	KM283849	
L. wallacei CBS101237	AY184967	AY184978	EF469073	EF469102	EF469119	EF641891
Simplicillium lanosoniveum CBS 704.86	AF339553	AF339602	DQ522358	DQ522406	DQ522464	AJ292396

Figures

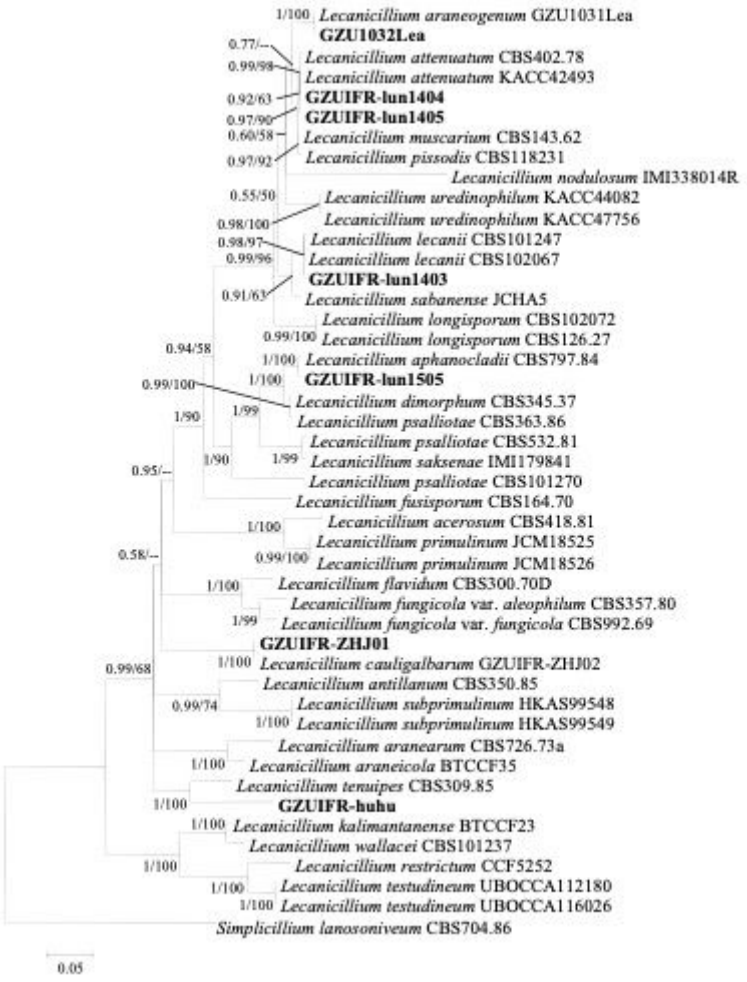

Figure 1

Phylogenetic analysis of the isolated strains and related species deriving from partial ITS+TEF+RPB1+RPB2 sequences. Statistical support values (≥ 0.5/50%) are shown at the nodes for BI posterior probabilities/ML boostrap support.

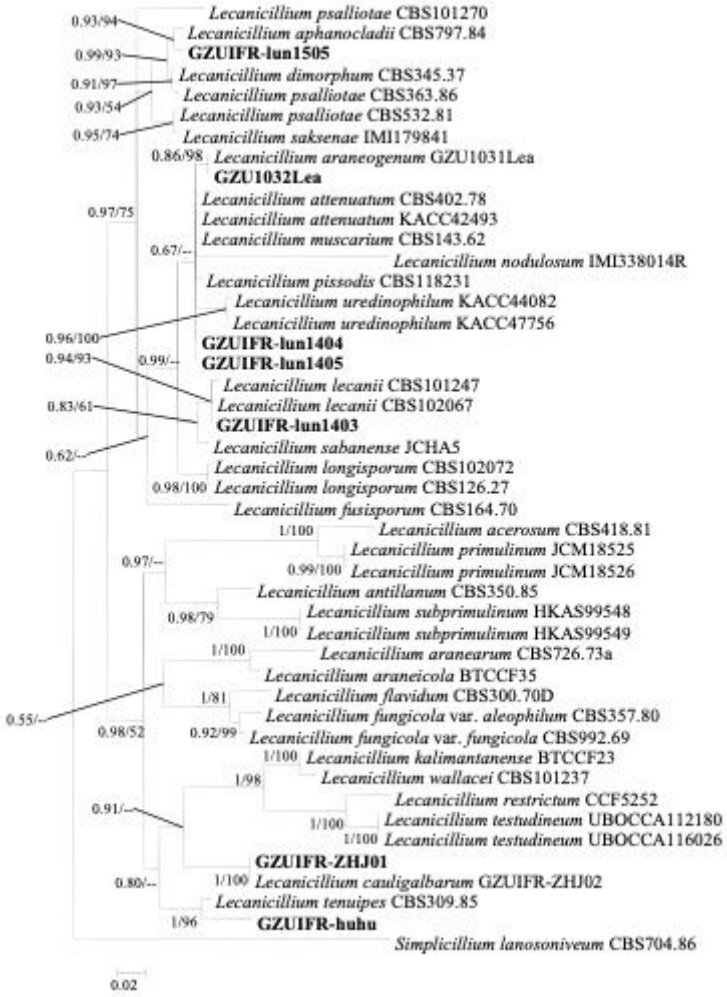

Figure 2

Phylogenetic analysis of the isolated strains and related species deriving from partial ITS +TEF sequences. Statistical support values (≥ 0.5/50%) are shown at the nodes for BI posterior probabilities/ML boostrap support.

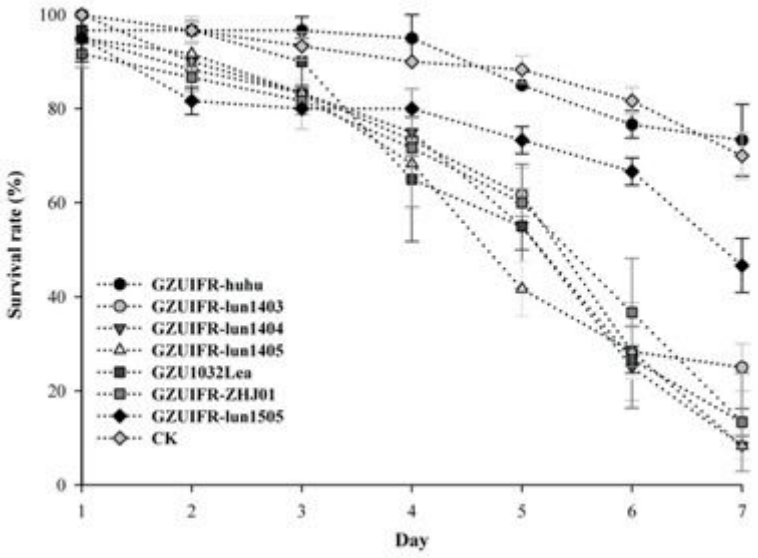

Figure 3

Survival rate (%) of F. occidentalis adult after inoculating with different fungal species. (mean ± SE).

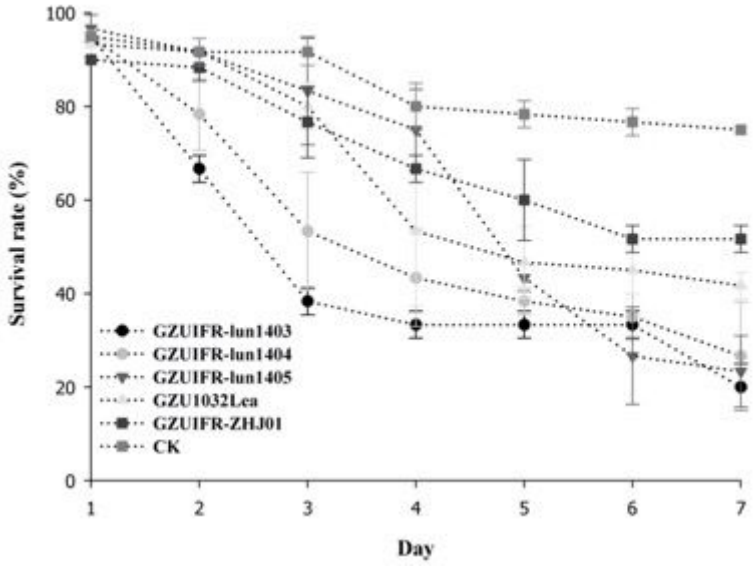

Figure 4

Survival rate (%) of F. occidentalis second instar nymph after inoculating with different fungal species. (mean ± SE).

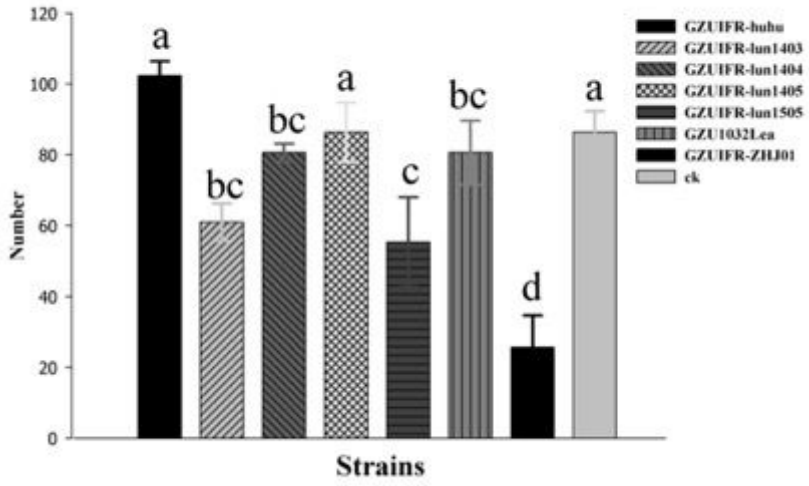

Figure 5

The number of nymphs appeared by the F. occidentalis adult females after the bioassay experiment.

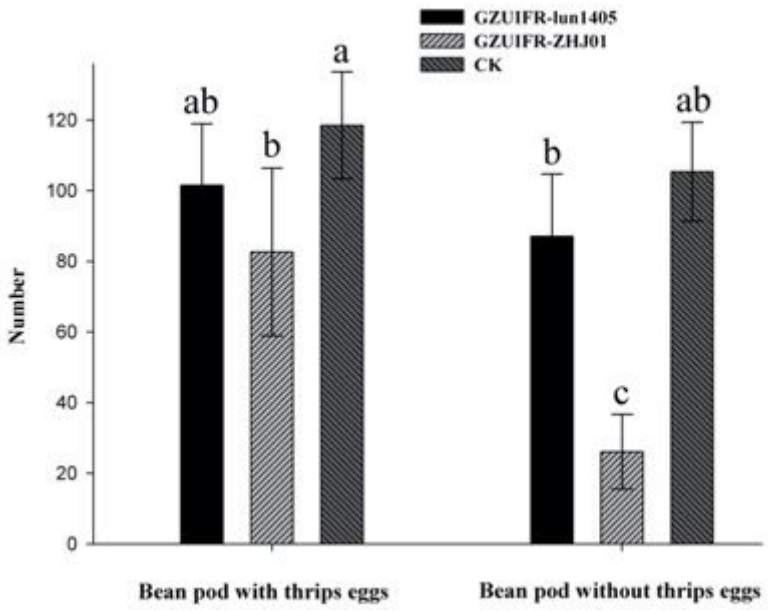

Figure 6

The numbers of nymph of F. occidentalis appeared under different treatments.

Supplementary Files

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- SupplementaryMaterial.docx