

环境因子和苹果品种对 *Alternaria mali* 强弱毒菌株致病活性的影响

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摘要:【目的】明确不同环境因子(温度、相对湿度、光照)和苹果品种对 *Alternaria mali* 强弱毒菌株致病活性的影响。【方法】采用喷雾接种法评价不同环境因子和苹果品种对 *A. mali* 强弱毒菌株致病活性的影响。【结果】不同温度、相对湿度和光照条件对 *A. mali* 强弱毒菌株致病活性均产生了不同程度的影响, 整体程度上强毒菌株的潜育期短于弱毒菌株, 发病程度高于弱毒菌株。在不同温度条件下, 当温度为 30 °C 时, *A. mali* 强弱毒菌株潜育期均最短, 分别为 54 h 和 72 h; *A. mali* 强毒菌株在温度为 25 °C 时致病活性最强, 病情指数为 23.06, 而弱毒菌株在 30 °C 时致病活性最强, 病情指数为 17.53, 但是当温度为 15 °C 时, *A. mali* 强弱毒菌株潜育期最长, 均大于 100 h, 并在此温度下叶片发病最轻, 其病情指数分别为 5.86 和 8.42。在不同湿度条件下, 当相对湿度为 60% 时, *A. mali* 强弱毒菌株潜育期均最长, 为 120 h, 并且在此湿度条件下叶片的发病程度最轻, 病情指数分别为 10.88 和 9.42, 而当相对湿度达到 100% 时, 强弱毒菌株潜育期最短, 均为 72 h。在不同光照条件下, *A. mali* 强弱毒菌株均在光暗交替、紫外照射+持续光照条件下潜育期最短, 其中强毒菌株在光暗交替、紫外照射+持续光照条件下潜育期均为 60 h, 而弱毒菌株在此条件下均为 72 h, 但是在光暗交替条件下, 强毒菌株病情指数最高, 为 22.59, 而在紫外照射+持续光照下, 弱毒菌株病情指数最高, 为 21.24。此外, *A. mali* 强弱毒菌株对不同苹果品种的致病活性存在显著差异, 其中强弱毒菌株对富士和新红星品种致病性较弱, 而对金冠致病性最强。【结论】不同环境因子和苹果品种对 *A. mali* 强弱毒菌株致病活性具有显著影响, 研究结果可为苹果早期落叶病科学防治提供理论依据。

关键词: 苹果叶斑病; *Alternaria mali*; 强弱毒菌株; 潜育期; 致病性

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Effect of environmental factors and apple varieties on the pathogenic activity of virulent and attenuated *Alternaria mali* strains

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Abstract: 【Objective】The apple early leaf blight disease is one of the major diseases caused by the pathogen of *Alternaria mali*, which has widely distributed in the main apple-producing regions worldwide and posed a serious threat to both the yield and quality of apple. This disease is caused by *A. mali* strains with varying degrees of virulence. Virulent *A. mali* strain leads to spot leaf blight disease, which affects leaves and younger shoots, influencing the growth of flower buds and fruit. Attenuated *A. mali* strain causes the target spot disease, which mainly affects the leaves and results in large lesions on the fruit. Environmental factors, especially temperature and humidity, play the significant role in impacting the disease occurrence. The aims for the present study were to clarify the effects of different environmental factors (temperature, relative humidity and light conditions) and apple cultivars on the latent period

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and pathogenicity of both virulent and attenuated *A. mali* strains. **【Methods】** In this study, the spray inoculation method was used to evaluate the effects of different environmental factors and apple varieties on the pathogenic activity of virulent and attenuated strains of *A. mali*. Healthy apple branches with consistent growth and diameter were collected and placed into sterile flasks containing sterile water, with five branches per flask. The virulent and attenuated strains of *A. mali* were inoculated by spraying spore suspensions (1×10^5 conidia \cdot mL⁻¹) onto the front and back of leaves growing on the branches, with sterile water as the control. In the temperature experiment, the treatment and control groups were cultured with a relative humidity of 90% and at temperatures of 15 °C, 20 °C, 25 °C, 30 °C and 35 °C (16 h light/8 h dark), with three repetitions for each group. In the humidity experiment, the inoculated branches were cultured at 25 °C under 16 h light/8 h dark conditions, and the relative humidity was adjusted by modifying the concentration of H₂SO₄ to 60%, 70%, 80%, 90% and 100%. In the light condition experiment, the inoculated branches were placed at 25 °C and 90% humidity and exposed to various light treatments: continuous light, continuous darkness, 12 h light/12 h dark cycles, 3 h UV irradiation and 21 h light. In the variety experiment, branches of Starkrimson, Fuji and Golden Delicious were placed in sterile water, and the *A. mali* spore suspension was sprayed onto the leaves, with three repetitions for each variety, and the branches were cultured at 25 °C with 90% humidity under 16 h light/8 h dark conditions. After inoculation, the incubation period was observed every 6 hours and the disease index were recorded after 7 days. **【Results】** Different temperatures, relative humidity and light conditions all exerted varying degrees of influence on the pathogenic activity of virulent and attenuated strains of *A. mali*, with the virulent strain exhibiting a significantly shorter incubation period and higher disease severity than the attenuated strain averagely. Under different temperature conditions, at 30 °C, both the virulent and attenuated strains of *A. mali* had the shortest incubation periods, being 54 h and 72 h, respectively. Within the temperature range from 20 °C to 30 °C, disease severity was more pronounced after inoculation, with the virulent strain showing the highest pathogenic activity at 25 °C, where the disease index reached 23.06 and the attenuated strain showing the highest activity at 30 °C, with a disease index of 17.53. However, at 15 °C, the incubation period was the longest for both strains, exceeding 100 h and the disease severity on the leaves was the slightest, with disease index of 5.86 and 8.42, respectively. Under different humidity conditions, with 60% relative humidity, the incubation period for both strains was the longest, 120 h, while at 100% relative humidity, the incubation period was the shortest, only 72 h. Additionally, with 60% relative humidity, the disease index for both strains were the lowest, being 10.88 and 9.42, respectively, while with 90% relative humidity, the disease index were the highest, being 19.01 and 12.50. Under different light conditions, both strains had the shortest incubation periods under alternating light/dark cycles, UV irradiation and continuous light. The virulent strain had a 60 h incubation period under these conditions, while the attenuated strain had a 72 h incubation period. However, under alternating light/dark cycles, the virulent strain had the highest disease index of 22.59, while under UV irradiation and continuous light conditions, the attenuated strain had the highest disease index of 21.24. Moreover, significant differences were observed in the pathogenic activity of the virulent and attenuated strains among different apple varieties. The pathogenicity of both strains on Fuji was generally lower than that on Starkrimson and Golden Delicious, with disease index of 14.13 and 8.30, respectively. Compared to the virulent and attenuated strain pathogenicity on Starkrimson, their pathogenicity on Golden Delicious was stronger, with the highest disease index being 16.82 and 22.09, respectively. The disease resistance evaluations showed that Fuji exhibited moderate resistance to the attenuated strain of *A. mali* and resistance to the virulent strain, while Starkrimson and Golden Delicious both displayed resistance to both strains.

【Conclusion】 Different temperatures, relative humidity, light conditions and apple cultivars had varying degrees of influence on the incubation period and pathogenicity of virulent and attenuated strains of *A. mali*. It was found that when the temperature was between 25–30 °C, relative humidity was 90%–100% and the light conditions included alternation of light and darkness, UV irradiation and continuous light, the incubation period of *A. mali* virulent and attenuated strains on Starkrimson was shorter, and pathogenicity was stronger. Additionally, Fuji showed better disease resistance to these strains. These findings can provide a theoretical basis for the scientific and rational control of apple early leaf blight disease.

Key words: Apple leaf spot; *Alternaria mali*; Virulent and attenuated strains; Incubation period; Pathogenicity

苹果 (*Malus pumila* Mill.) 为蔷薇科 (Rosaceae) 苹果属 (*Malus*) 落叶乔木, 因其果实具有营养价值高、耐贮性好和供应周期长等特点, 已成为农民增收致富的支柱产业之一^[1]。然而, 苹果早期落叶病在国内苹果主产区广泛发生, 给苹果产业高质量发展带来了挑战^[2-3]。该病害发生后常导致苹果树提早大量落叶, 严重削弱树势, 发生严重情况下造成当年或翌年果实品质及产量下降^[4]。相关研究表明, 苹果早期落叶病种类主要包括苹果褐斑病、斑点落叶病、轮斑病、圆斑病、灰斑病和炭疽叶枯病等, 并且不同区域的优势病害种类不同, 其中苹果褐斑病和苹果斑点落叶病较为常见^[5-7]。链格孢菌 (*Alternaria* spp.) 是引起多种苹果早期落叶病的重要病原之一^[8]。Harteveld 等^[9]在澳大利亚发现引起苹果链格孢叶斑病和果斑病的主要病原为 *A. longipes*、*A. arborescens*、*A. alternata*/*A. tenuissima* 和 *A. tenuissima*/*A. mali*, 且同一物种内分离株在致病性和毒力方面表现出显著变异和交叉致病性; Toome-Heller 等^[10]首次在新西兰发现苹果链格孢复合种 (*A. arborescens*) 可引起苹果叶斑病且出现褐斑病症状。何劲等^[11]研究发现, 贵州地区苹果早期落叶病种类主要有苹果轮斑病和斑点落叶病, 其病原分别为 *A. mali* 和 *A. alternata*, 其中, 由交链格孢 (*A. alternata*) 引起的苹果斑点落叶病是苹果生产中危害严重的病害之一, 在全球苹果主产区均有发生^[12]。然而, 也有相关研究发现, 苹果斑点落叶病和苹果轮斑病可由 *A. mali* 的不同毒力菌株引起^[13-15], 其中 *A. mali* 弱毒菌株引起的苹果轮斑病主要危害叶片, 也可危害果实且病斑较大, 该病害流行时植株发病率可达 100%^[16], 而 *A. mali* 强毒菌株主要危害苹果叶片和嫩枝, 可引起苹果斑点落叶病, 发生后影响花芽形成和果实正常生长^[17], 导致叶部出现褐色病斑和树势衰弱, 病害流行时引起 70% 苹果树

叶片早期脱落^[18]。

邵旭平等^[19]将引起甘肃省苹果斑点落叶病的病原鉴定为 *A. mali* 的强毒菌株, 苹果轮斑病的病原鉴定为 *A. mali* 的弱毒菌株, 并发现 *A. mali* 强弱毒菌株间具有交叉保护作用, 可有效降低病害的发生。同时, 苹果斑点落叶病的发生和蔓延与环境因素密切相关, 特别是温度和湿度可以通过影响苹果斑点落叶病菌 (*A. mali*) 孢子的萌发来调控病菌的生长, 在 30 °C 和 100% 相对湿度下, 孢子萌发率最高, 而在极端温湿度条件下, 萌发率显著下降^[20]。此外, 另有研究发现在 9 种不同温度 (4~36 °C) 和 8 种保湿时间 (2~48 h) 的组合条件下, *A. mali* 均能感染苹果幼苗, 且随着保湿持续时间的延长, 病害发生的程度显著加剧^[21]。但是, 目前有关 *A. mali* 强弱毒菌株在不同环境因素下的侵染规律、发病条件及苹果品种对其抗性方面缺乏全面系统的研究。鉴于此, 笔者以课题组前期分离鉴定的 *A. mali* 强弱毒菌株作为供试菌株, 采用离体叶片接种测定不同环境因子 (温度、相对湿度和光照) 和不同苹果品种对 *A. mali* 强弱毒菌株潜育期及致病力强弱的影响, 以为苹果早期落叶病的防控提供理论支撑。

1 材料和方法

1.1 材料

1.1.1 供试菌株及其孢子悬浮液制备 供试 *A. mali* 强弱毒菌株均保存于甘肃农业大学植物保护学院植物病毒学与分子生物学实验室。参考 Harimoto 等^[22]的方法配制浓度为 1×10^5 个 $\cdot \text{mL}^{-1}$ 的 *A. mali* 强弱毒菌株孢子悬浮液, 备用。

1.1.2 供试苹果品种 供试苹果品种分别为新红星、富士和金冠。选择长势和粗细一致的健康苹果枝条作为室内离体接种试材, 均采集自兰州市七里河区苹果种植基地, 树龄为 18~21 a (年)。

1.2 方法

1.2.1 不同温度对 *Alternaria mali* 强弱毒菌株致病活性的影响 将采集的长势和粗细一致的新红星健康苹果枝条置于装有灭菌水的无菌三角瓶内,每瓶5枝,采用喷施接种法将 *A. mali* 强弱毒菌株孢子悬浮液(1×10^5 个 \cdot mL $^{-1}$)分别接种于供试枝条叶片的正面和反面,并以接种等体积灭菌水作为对照。随后,将各处理和对照分别置于塑料罩内以保持湿度(相对湿度90%),并分别置于15、20、25、30和35℃的人工气候箱(16 h光照/8 h黑暗)内培养,并待接种后每隔培养6 h观察和记录潜育期。同时,待接种7 d后,统计叶片病情指数。试验过程中不同温度处理下,每个处理和对照均重复3次。

参照崔琳霞等^[23]和王程亮等^[24]分级标准进行分级。具体分级如下:0级,叶片上未观察到斑点;1级,斑点覆盖面积占叶片总面积比例小于10%;3级,斑点覆盖面积占叶片总面积比例为11%~25%;5级,斑点覆盖面积占叶片总面积比例为26%~40%;7级,斑点覆盖面积占叶片总面积比例为41%~65%;9级,斑点覆盖面积占叶片总面积比例大于66%。

$$\text{病情指数} = \frac{\sum(\text{病级叶片数} \times \text{病级代表值})}{\text{调查总叶数} \times 9} \times 100。$$

1.2.2 不同相对湿度对 *Alternaria mali* 强弱毒菌株致病活性的影响 将按上述接种方法处理后的枝条分别置于干燥器中,利用不同浓度的H₂SO₄调整并设置相对湿度分别为60%、70%、80%、90%和100%。然后,置于温度为25℃和光照条件为16 h光照/8 h黑暗的培养箱中培养,并待接种后每隔培养6 h观察和记录潜育期。待接种7 d后,统计叶片病情指数。试验每个处理和对照均设置3个重复。

1.2.3 不同光照条件对 *Alternaria mali* 强弱毒菌株致病活性的影响 将经上述接种处理后的枝条分别置于不同光照[持续光照(24 h \cdot d $^{-1}$)、持续黑暗(24 h \cdot d $^{-1}$)、光照与黑暗交替(12 h光照/12 h黑暗)、紫外照射3 h和光照处理21 h]、温度为25℃和相对湿度为90%条件下培养,并待接种后每隔培养6 h观察和记录潜育期。待接种7 d后,统计和计算叶片的病情指数。试验每个处理和对照均设置3个重复。

1.2.4 不同品种对 *Alternaria mali* 强弱毒菌株致病活性的影响 将采集的长势和粗细一致的健康新红星、富士及金冠3个品种枝条分别置于装有灭菌水的无菌三角瓶内,每瓶5枝。然后,采用喷施接种

法将 *A. mali* 强弱毒菌株孢子悬浮液(1×10^5 个 \cdot mL $^{-1}$)分别接种于不同品种供试枝条叶片的正反面,并以接种等体积灭菌水作为对照,每个处理和对照均设置3次重复。然后,将其放置在温度为25℃、相对湿度为90%、光照条件为16 h光照/8 h黑暗的环境中培养。待接种后每隔培养6 h观察和记录潜育期,并待接种7 d后,统计叶片病情指数。同时,参照王昆等^[25]抗病性评价标准对不同品种进行抗病性评价。病情指数(DI) ≤ 5 ,高抗;5<DI ≤ 10 ,中抗;10<DI ≤ 30 ,抗病;30<DI ≤ 50 ,感病;DI>50,高感。

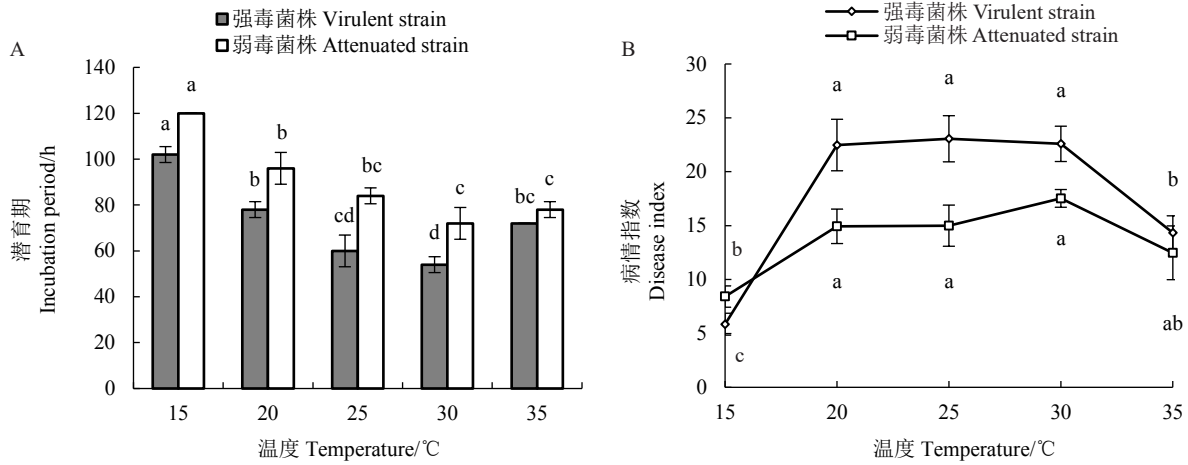
2 结果与分析

2.1 不同温度对 *Alternaria mali* 强弱毒菌株致病活性的影响

不同温度对 *A. mali* 强弱毒菌株潜育期和病情指数均具有不同程度的影响。随着温度升高, *A. mali* 强弱毒菌株潜育期呈先降低(15~30℃)后升高(30~35℃)的变化趋势(图1-A),而病情指数表现出先升高后降低的变化趋势(图1-B)。在不同温度条件下, *A. mali* 弱毒菌株的叶片潜育期始终较 *A. mali* 强毒菌株长,当温度条件为30℃时, *A. mali* 强弱毒菌株的潜育期均最短,分别为54 h和72 h;在温度为20~30℃范围时, *A. mali* 强弱毒菌株接种叶片后,发病程度较为严重,其中接种强弱毒菌株后, *A. mali* 强毒菌株在温度为25℃时致病活性最强,病情指数为23.06,而弱毒菌株在30℃时致病活性最强,病情指数为17.53。然而,当温度为15℃时, *A. mali* 强弱毒菌株潜育期均大于100 h,并在此温度下接种 *A. mali* 强弱毒菌株后,接种 *A. mali* 强毒菌株的叶片发病严重程度显著低于接种弱毒菌株的叶片,病情指数分别为5.86和8.42。

2.2 不同相对湿度对 *Alternaria mali* 强弱毒菌株致病活性的影响

由图2-A可知,与 *A. mali* 弱毒菌株相比, *A. mali* 强毒菌株在叶片上的潜育期整体较短,并且随着相对湿度(60%~100%)的增加, *A. mali* 强毒菌株在叶片上的潜育期呈逐渐变短到趋于稳定的趋势,而弱毒菌株呈先趋于稳定后逐渐变短的趋势。当相对湿度为60%时, *A. mali* 强弱毒菌株的潜育期最长,均为120 h,而当相对湿度达到100%时,潜育期均达到最短,为72 h。由图2-B可知,在不同相对湿度条件下培养7 d后,发现接种 *A. mali* 弱毒菌株的叶片发病程度低于 *A. mali* 强毒菌株,并且在60%的相对湿度条



不同小写字母表示在 0.05 水平差异显著。下同。

Different small letters represent significant difference at 0.05 level. The same below.

图 1 不同温度对 *A. mali* 强弱毒菌株潜育期 (A) 和致病活性 (B) 的影响

Fig. 1 Effects of different temperatures on incubation periods (A) and pathogenic activity (B) of virulent and attenuated strains of *A. mali*

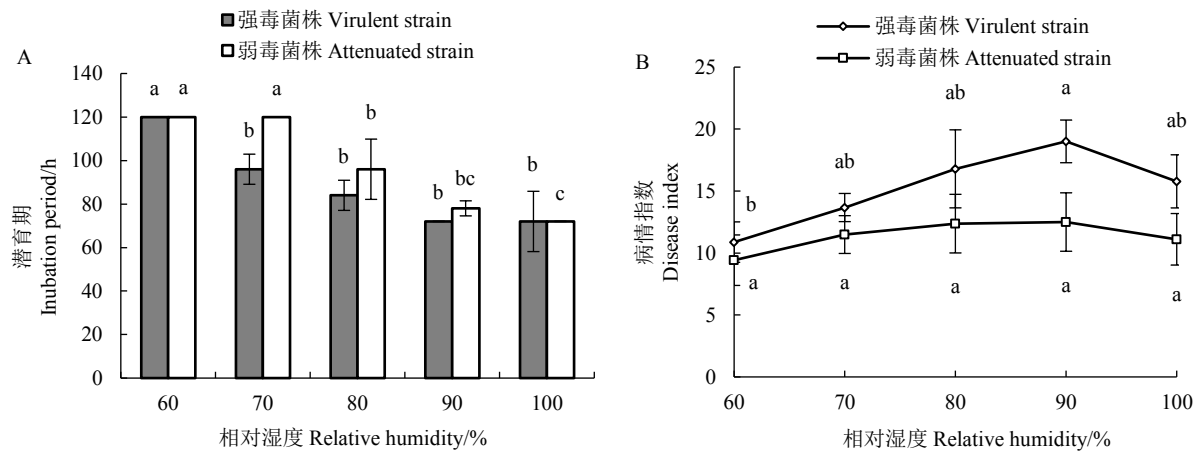


图 2 不同相对湿度对 *A. mali* 强弱毒菌株潜育期 (A) 和致病活性 (B) 的影响

Fig. 2 Effects of different relative humidity on incubation periods (A) and pathogenic activity (B) of virulent and attenuated strains of *A. mali*

件下,接种强弱毒菌株后的叶片病情指数均最低,分别为 10.88 和 9.42,而在 90% 的相对湿度条件下,接种 *A. mali* 强弱毒菌株后的叶片病情指数均最高,分别为 19.01 和 12.50。

2.3 不同光照条件对 *Alternaria mali* 强弱毒菌株致病活性的影响

在持续光照、持续黑暗、光暗交替、紫外照射+持续光照条件下, *A. mali* 强毒菌株的潜育期均较 *A. mali* 弱毒菌株短,并且在光暗交替、紫外照射和持续光照条件下,强弱毒菌株潜育期较短,其中强毒菌株在光暗交替、紫外照射+持续光照条件下潜育期均为 60 h,而弱毒菌株在此条件下均为 72 h(图 3-A)。在不同光照条件下培养 7 d 后,除紫外照射+持续光照

条件外,在其他不同光照处理下, *A. mali* 强毒菌株接种叶片后,叶片的病情指数均高于弱毒菌株,其中 *A. mali* 强毒菌株在光暗交替条件下病情指数最高,为 22.59,但是在紫外照射+持续光照条件, *A. mali* 弱毒菌株侵染叶片后病情指数最高,为 21.24。然而, *A. mali* 强弱毒菌株均在持续黑暗条件下,接种叶片后病情指数最低,分别为 13.58 和 13.33(图 3-B)。

2.4 不同品种对 *Alternaria mali* 强弱毒菌株致病活性的影响

不同苹果品种叶片接种 *A. mali* 强弱毒菌株后, *A. mali* 强弱毒菌株在新红星和金冠品种上的潜育期均显著低于富士品种,并且强弱毒菌株均在金冠品种上潜育期最短且相同(48 h)(图 4-A)。待接种 *A. ma-*

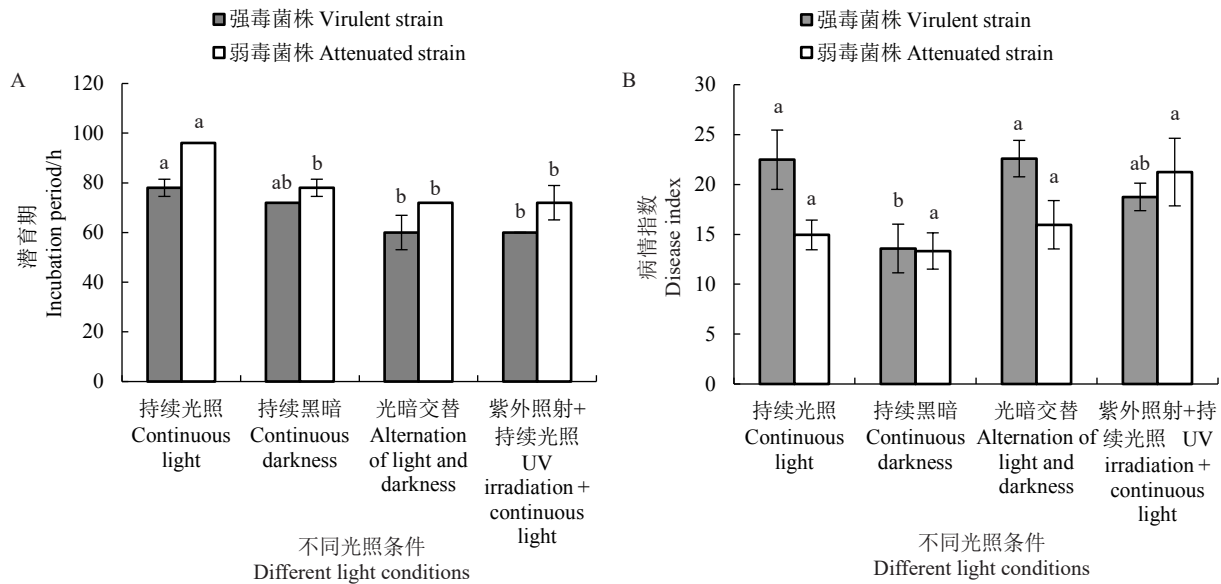


图 3 不同光照条件对 *A. mali* 强弱毒菌株潜育期 (A) 和致病活性 (B) 的影响

Fig. 3 Effects of different light intensity on incubation periods (A) and pathogenic activity (B) of virulent and attenuated strains of *A. mali*

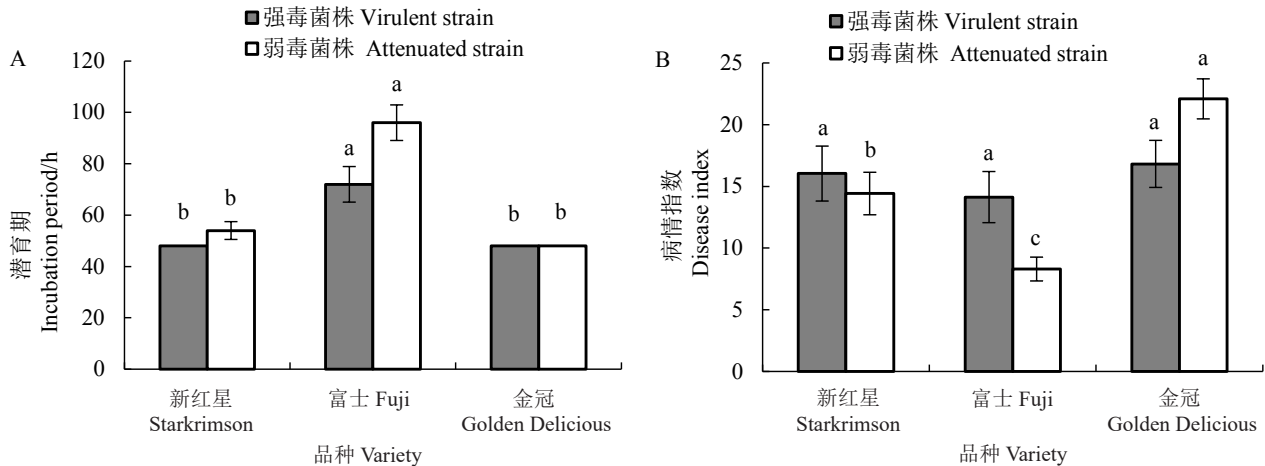


图 4 不同苹果品种对 *A. mali* 强弱毒菌株潜育期 (A) 和致病活性 (B) 的影响

Fig. 4 Effects of different apple varieties on incubation periods (A) and pathogenic activity (B) of virulent and attenuated strains of *A. mali*

li 强弱毒菌株 7 d 后, *A. mali* 强弱毒菌株对富士品种的致病力整体低于新红星和金冠品种, 其中在富士品种上的病情指数分别为 14.13 和 8.30。与 *A. mali* 强弱毒菌株对新红星的致病力相比, 其对金冠的致病力较强, 病情指数最高, 分别为 16.82 和 22.09 (图 4-B)。不同品种抗病性评价结果表明, 富士品种对 *A. mali* 弱毒菌株表现为中抗, 而对 *A. mali* 强毒菌株表现为抗病, 但是新红星和金冠对 *A. mali* 强弱毒菌株均表现为抗病。

3 讨 论

笔者通过测定不同环境 (温度、相对湿度和光照) 对 *A. mali* 强弱毒菌株潜育期和致病活性的影响, 发现在不同的温度、相对湿度和光照条件下, *A. mali* 强毒菌株的潜育期整体上较 *A. mali* 弱毒菌株短, 并且强毒菌株接种后的苹果叶片发病程度整体上高于弱毒菌株。吴桂本等^[26]研究表明, 胶东地区 *A. alternata* f. sp. *mali* 菌株 A1 致病性明显弱于 A2。研究表明, *A. mali* 强毒菌株接种印度苹果品种叶片后, 在温度为 25~30 °C 时, 其潜育期最短为 48 h, 病叶率最高为 90%^[27]。本研究表明, *A. mali* 强弱毒菌株接种新红星苹果品种叶片后, 在温度为 30 °C 时

潜育期最短,且在温度为20~30℃范围时,叶片发病程度较为严重,尤其温度为25℃和30℃。然而,在温度为25℃、相对湿度为90%条件下,*A. mali* 强毒菌株接种苹果叶片后,其潜育期最短且病情指数最高,这一结果与胡同乐等^[28]关于苹果斑点落叶病在降雨后,相对湿度为90%且持续10 h以上会导致病原菌大量侵染的研究结果相吻合。此外,与持续光照相比,在12 h光照与12 h黑暗交替、3 h紫外线照射和21 h持续光照条件下,*A. mali* 强弱毒菌株在叶片上的潜育期均显著缩短,病情指数均达到最高峰,初步发现紫外线照射可增强*A. mali* 弱毒菌株的致病性,进而促进了病斑的形成速度。薛军等^[29]研究表明,苹果斑点落叶病的发病条件与田间光照时长及紫外线辐射强烈相关,光照时长及紫外线辐射可促使病原菌在叶片上的潜育期缩短,从而促进病害的发生,与本试验室内条件下的研究结果一致。

吕松等^[30]发现在新疆的野生苹果和引进的西洋苹果上苹果斑点落叶病的发病率较高,而在国产苹果和野生品种中,发病率则显著降低。本试验结果表明,*A. mali* 强弱毒菌株在金冠品种上的潜育期最短且致病性最强,与徐秉良^[27]研究发现致病力较强的*A. mali* 菌株(兰州1号)对金冠品种致病活性较强的结果一致。另外,红星、印度和金冠等苹果品种在接种*A. mali* 后,其中红星和印度均为高感品种,金冠为中抗品种^[27],而本试验初步发现富士对*A. mali* 强弱毒菌株分别表现为抗病和中抗,新红星和金冠对强弱毒菌株均表现为抗病。因此,本试验明确了不同环境因子和苹果品种对*A. mali* 强弱毒菌株致病活性具有显著影响,而有关不同环境因子和苹果品种对*A. mali* 强弱毒菌株潜育期和致病性影响的机制还有待进一步深入研究。

4 结 论

不同温度、相对湿度、光照条件及苹果品种对*A. mali* 强弱毒菌株潜育期和致病性具有不同程度的影响,当温度为25~30℃、相对湿度为90%~100%、光照条件为光暗交替、紫外照射+持续光照时,*A. mali* 强弱毒菌株在新红星品种上的潜育期较短,致病性较强。富士苹果对*A. mali* 强弱毒菌株均具有较强抗病性,分别表现为抗病和中抗。

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