Research Progress and Challenges of Tobacco Brown Spot

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Abstract This paper provide a comprehensive overview of research findings regarding the pathogen responsible for tobacco brown spot, its occurrence regularity, and integrated control strategies. Additionally, this study provide a brief analysis of the challenges encountered in the study of tobacco brown spot, which include the study of pathogenesis and virulence, the breeding of disease-resistant varieties, the screening of low-toxicity and high-efficiency agents, the development of biological control methods with more stable efficacy, and the necessity for accurate prediction and forecasting techniques.

Key words Tobacco brown spot, Integrated control, Challenge

1 Introduction

Tobacco brown spot is the most significant fungal leaf disease affecting tobacco during its maturation. This disease is characterized by intermittent outbreaks, a short incubation period, and a rapid onset. Under favorable environmental conditions, outbreaks can occur over extensive areas in a short time, resulting in significant economic losses in severe cases. The infection of tobacco brown spot can alter the chemical composition of tobacco leaves, which may significantly impact the quality of tobacco leaves. This, in turn, affects the quality of cigarette products and diminishes the overall utility of tobacco leaves. Tang Yuanman *et al.*[1] observed that following infection by tobacco brown spot, the total nitrogen content in both the lower and upper leaves increased in correlation with the level of infection. Conversely, the levels of total sugar, reducing sugar, and starch exhibited a decline as the infection level increased.

2 Overview of tobacco brown spot

2.1 Pathogen The pathogen responsible for tobacco brown spot is classified within the genus *Alternaria*, which is part of the subphylum Deuteromycotina. Currently, the identified causative agents of tobacco brown spot include *Alternaria alternata* (Fr.: Fr.) keissler, *A. longipes* (Ell. & Ev.) Mason, *A. tenuissima* (Nees & T. Nees: Fr.) Wiltshire, *A. yaliinficiens* R. G. Roberts, *A. tabacicola* Y. Q. Zu & M Zhang, and *A. nicotianae* Cheng et An[2-3]. Cheng Julong *et al.*[4] identified a novel pathogenic fungus, *A. nicotianae* Cheng et A n, in Luonan and Qianyang through the analysis of 300 samples of tobacco brown spot collected from 12 counties in Shaanxi. Zu Yanqing *et al.*[5] conducted a morphological and molecular identification of the fungus responsible for tobacco brown spot, which was isolated from six cities in Henan Province, adhering to internationally recognized standard methods. The study identified that the fungi causing tobacco brown spot in Henan include *A. nicotianae*, *A. longipes*, and *A. yaliinficiens*. Notably, this research marks the first report of *A. yaliinficiens* as a causative agent of tobacco brown spot. Zu Yanqing[3] isolated and identified the fungus responsible for tobacco brown spot in seven cities within the tobacco-growing region of Henan Province. In addition to identifying *A. nicotianae*, *A. longipes*, *A. tenuissima*, and *A. yaliinficiens*, the study also led to the discovery of a new microspore species within the genus *Alternaria*, designated as *A. tabacicola* Y. Q. Zu & M. Zhang.

There are significant regional variations in the predominant pathogenic species responsible for tobacco brown spot. Niu Junke *et al.*[6] conducted a study in which they isolated and identified the fungus responsible for tobacco brown spot from 15 tobacco-growing regions in Jilin Province and 7 tobacco-growing regions in Heilongjiang Province. Through both morphological and molecular biological identification techniques, they identified three species in the tobacco-growing region of Jilin Province: *A. nicotianae*, *A. tenuissima*, and *A. yaliinficiens*. In contrast, the tobacco-growing regions of Heilongjiang Province yielded three species: *A. longipes*, *A. nicotianae*, and *A. tenuissima*. Notably, *A. nicotianae* emerged as the dominant species in both provinces. Yang Tao[2] identified that the fungi responsible for tobacco brown spot in four tobacco-growing regions of Hubei included *A. nicotianae*, *A. tenuissima*, *A. longipes*, and *A. yaliinficiens*. Notably, *A. longipes* was found to be the predominant species, comprising 67% of the total fungal population. Peng Xiwen *et al.*[7] identified the fungi responsible for tobacco brown spot in Yunnan Province as *A. nicotianae* and *A. longipes*, with the latter being the predominant species. Geng Lina *et al.*[8] isolated a dominant pathogenic strain responsible for tobacco brown spot in the Wuxi tobacco-growing region of Chongqing. This strain was sequenced using rDNA-ITS region sequences and identified as *A. tenuissima*, marking the first recorded instance of *A. tenuissima* in Chongqing tobacco-growing regions.

2.2 Symptoms Tobacco brown spot is the primary disease affecting tobacco plants during the maturity stage. This condition

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typically initiates on the lower leaves, with the spots progressively developing from the bottom upwards as the leaves mature. At the onset of the disease, the lesions present as small, yellow-brown, round spots. These spots subsequently darken to a brown hue and progressively enlarge, reaching diameters of 1 – 2 cm. The lesions may exhibit a round or irregularly round shape, characterized by distinct concentric whorls, with a yellowish halo surrounding the periphery. The dimensions of the spots are correlated with humidity levels, exhibiting larger spots in conditions of high humidity and smaller spots during periods of drought. The central region of the spots is characterized by the presence of dark brown or black mold. In severe instances, the spots may merge, resulting in scorched areas that detach from the leaf, ultimately leading to the complete degradation of the leaf blade and rendering it nonfunctional. Tobacco brown spot is characterized by the formation of dark brown, round or oblong, sunken lesions on both stalks and capsules.

2.3 Occurrence regularity The pathogen responsible for tobacco brown spot persists during the winter months as mycelia within infected plant debris, exhibiting enhanced overwintering efficacy in the affected stems. In the subsequent year, the mycelium produces conidia, which facilitate a primary infection via transmission. The pathogen subsequently proliferates on the tobacco plant, generating additional conidia that contribute to a secondary infection through further transmission. The pathogen is capable of dispersing over considerable distances aided by wind, as well as over shorter distances through rainfall.

Tobacco plants exhibit varying levels of resistance to pathogens throughout different growth stages. Chen Fawei *et al.* [9] reported that tobacco demonstrates a notable resistance to diseases during the seedling stage; however, as the plant matures, this resistance progressively diminishes, ultimately leading to a stage of heightened susceptibility at full maturity. The occurrence and progression of tobacco brown spot is significantly influenced by various factors, including the choice of planting varieties, cultivation practices, and meteorological conditions, with temperature and humidity being the most critical determinants. Li Tianfu *et al.* [10] identified that the relationship among temperature, humidity, sunshine hours, precipitation, and evapotranspiration in relation to tobacco brown spot can be categorized into two distinct phases. The first phase, characterized by high temperatures, low humidity, increased sunshine, reduced precipitation, and elevated evapotranspiration, is conducive to the development of tobacco brown spot. Conversely, the second phase, marked by low temperatures, high humidity, diminished sunshine, increased precipitation, and decreased evapotranspiration, also favors the occurrence of tobacco brown spot. Mo Jianguo *et al.* [11] identified that the pathogen responsible for tobacco brown spot in various climatic zones of Guizhou Province can infect tobacco leaves under conditions of 50% – 60% humidity and temperatures ranging from 10 to 20 ℃, which are characterized as low temperature and moderate humidity. Although some variations exist among the strains, these differences are not statistically significant.

3 Integrated control of tobacco brown spot

3.1 Agricultural control

3.1.1 Breeding disease-resistant varieties. Breeding disease-resistant varieties is a fundamental approach for mitigating the damage caused by tobacco brown spot and is also the most cost-effective solution to this issue. Sun Liping[12] utilized the toxin medium method to screen 1 229 materials from the EMS-induced Zhongyan 100 M2 mutant library for disease-resistant mutants against tobacco brown spot. Ultimately, six disease-resistant mutants were identified. Jiang Caihong *et al.* [13] discovered that the resistance of Beinhart 1000 – 1 to tobacco brown spot is governed by a dominant single gene. In contrast, the resistance of Jingyehuang to brown spot is not solely determined by a dominant single gene. They identified a marker, $S1118_{360}$, which is associated with the resistance gene of Beinhart 1000 – 1 against brown spot, exhibiting a genetic distance of 16.00 cM from the target gene. Additionally, a marker, $S2113_{340}$, linked to the resistance gene of Jingyehuang against brown spot was also identified, with a genetic distance of 12.88 cM from the target gene.

Screening germplasm resources with resistance against brown spot is a critical prerequisite for the breeding of disease-resistant varieties. Yu Haiqin[14] identified the resistance levels of nine primary cultivars of flue-cured tobacco in China. The study revealed that the varieties exhibiting resistance to moderate resistance against brown spot include Yunyin 100, while those demonstrating medium resistance include PVH19. Xu Bo *et al.* [15] identified field resistance to brown spot in 161 tobacco materials collected from various sources. They successfully screened 13 highly resistant varieties and 35 moderately resistant varieties. Notably, the varieties Liaoyan, Jiyan, and Longjiangyan, which were bred in Northeast China, exhibited moderate to high resistance to brown spot. In contrast, the varieties introduced from abroad generally demonstrated lower levels of resistance. Wang Yan[16] conducted a comparative analysis of 10 Heilongjiang sun-cured tobacco varieties to assess their resistance to brown spot. Among these varieties, Anxiaohuboxiang, Baoqingxiaohuboxiang, Daqingjin, Taikang cigar, and Jiaxin cigar exhibited no signs of brown spot infection throughout the entire reproductive period. However, following artificial inoculation, all varieties displayed disease indices approximately around 30.00, with the exception of Daqingye, which demonstrated a high level of resistance, while the others exhibited poor resistance. Kong Dejun *et al.* [17] conducted a systematic identification on the resistance to brown spot in 390 flue-cured tobacco germplasm resources sourced from various origins. Their findings revealed the presence of 10 local germplasm resources exhibiting resistance to brown spot in Guizhou Province, one variety bred in Guizhou Province that also demonstrated resistance, 10 introduced disease-resistant germplasm resources from other provinces, and 26 disease-resistant germplasm resources sourced from abroad.

3.1.2 Selecting disease-free seeds and cultivating disease-free robust seedlings. Tobacco seeds have the potential to carry the pathogen responsible for brown spot, which can result in the infection of tobacco seedlings. The utilization of sterilized seeds in the cultivation of robust seedlings has been shown to be an effective strategy for reducing the prevalence of brown spot. Sun Chuntai[18] observed that when neither tobacco seeds nor the seedling bed soil were subjected to sterilization, the incidence and disease index of tobacco brown spot in the field were recorded at 25.03% and 3.05, respectively. In contrast, following the sterilization of both seeds and seedling bed soil, the incidence and disease index of brown spot decreased to 9.03% and 1.26. These findings suggest that the sterilization of seeds and the cultivation of robust seedlings can significantly mitigate the impact of tobacco brown spot.

3.1.3 Strengthening cultivation management and applying fertilizers scientifically and reasonably. The cultivation of spring tobacco and the timely implementation of early transplanting can mitigate the peak occurrence of tobacco brown spot during the maturation period. It is essential to adopt a reasonable planting density to ensure that the leaves do not obstruct the ridges during the adult growth stage, thereby facilitating adequate ventilation. Additionally, an appropriate increase in the application of phosphorus and potassium fertilizers is recommended. This may include the application of a 1% solution of potassium dihydrogen phosphate, which should be administered three times during the resettling stage, vigorous growth stage, and topping stage. Furthermore, enhancing field hygiene is crucial for the thorough destruction of plant residues. Topped branches should be removed from the tobacco field and either buried deeply or sun-dried for effective disposal[19]. The timing of topping is associated with the severity of tobacco brown spot. Pu Yu *et al.*[20] observed that topping conducted during the budding stage resulted in the most severe damage from brown spot, with the disease progressing at the fastest rate. In contrast, topping performed at the initial flowering stage resulted in a lower incidence of the disease, while topping at the full blooming stage led to the least incidence and the slowest progression of the disease. Notably, the disease index for topping at the full blooming stage was reduced by 5.7% compared to topping at the budding stage. The pathogen responsible for tobacco brown spot has the capability to persist in the soil for over one year. Implementing an effective crop rotation strategy that includes gramineous crops or sweet potatoes can significantly diminish the population of pathogenic fungi in the soil. Wu Zhekuan *et al.*[20] conducted a comparative analysis of four different crop rotation systems and determined that the rotation involving flue-cured tobacco and corn, followed by flue-cured tobacco, was the most effective. This particular rotation not only enhances the agronomic characteristics of flue-cured tobacco but also improves root vigor. Scientific and rational fertilization is a crucial strategy for managing tobacco brown spot. Research conducted by Fan Jingxiu *et al.*[21] indicates that an increase in nitrogen application correlates with a rise in the incidence of tobacco brown spot. In Shennongjia tobacco-growing region, the optimal ratio of nitrogen, phosphorus, and potassium is suggested to be 1.0 : 1.2 : 3.0 or 1.0 : 1.5 : 3.0. Furthermore, the recommended application dosage of pure nitrogen fertilizer typically ranges from 67.5 to 90.0 kg/hm^2. Chen Li[22] observed that an increase in nitrogen application, coupled with a higher number of leaves retained in areas endemic to brown spot, significantly contributed to the outbreak of this disease. This phenomenon resulted in a substantial decrease in the actual yield when compared to the theoretical yield in treatments characterized by high nitrogen levels and a greater number of retained leaves. Conversely, there was an insignificant increase in actual yield in treatments with normal nitrogen application and a standard number of leaves retained.

3.2 Chemical control Chemical control is presently regarded as the most significant method against tobacco brown spot, as it is more rapid and effective compared to other methods. Zeng Chaoning[23] conducted a comparative analysis of the induced resistance effects of six resistance inducers: salicylic acid, methyl jasmonate, benzothiadiazole, dichloroisonicotinic acid, chitin, and oxalic acid, on tobacco brown spot. The study revealed that the same inducer exhibited varying induced resistance effects against brown spot at different concentrations. Furthermore, the efficacy of different resistance inducers in preventing and controlling brown spot varied at their respective optimal treatment concentrations. Notably, benzothiadiazole demonstrated the most effective prevention and control, achieving relative efficacy rates of 62.2% and 57.6% during the peak and late stages of the disease, respectively. Li Hongguang *et al.*[24] conducted field control trials involving seven agents during the late growth stage of tobacco. The results indicated that both 40% dimetachlone-copper oxychloride WP and 40% dimetachlone WP exhibited superior relative efficacy, with the effectiveness of three applications exceeding 70%. The average efficacy was recorded at 75.81%, demonstrating stability in performance, and no phytotoxicity was observed in the tobacco plants. Xiao Qingling *et al.*[25] conducted efficacy tests on six chemical agents and determined that three of these agents, 40% dimethachlon, 45% dimetachlone Hu ketone, and 40% sclerotium · cuprum WP, demonstrated superior effectiveness in controlling tobacco brown spot, achieving efficacy rates of 76.29%, 74.12%, and 76.80%, respectively. Liu Dan[26] reported that the eight agents utilized in the experiment exhibited varying degrees of inhibition on the mycelial growth of tobacco brown spot at the tested concentrations. Notably, 10% difenoconazole was identified as the most effective agent, with an EC_{50} value of 7.45 μg/mL. Furthermore, at a concentration of 100 μg/mL, 10% difenoconazole demonstrated an inhibition effect of 86.67%. Zhu Yuhang *et al.*[27] investigated the indoor toxicity and field efficacy of fluazinam, picoxystrobin, boscalid, cyprodinil, and famoxadone in combating brown spot. Their findings indicated that a concentration of 98% fluazinam exhibited the most potent inhibitory effect on the mycelial growth of the pathogen, with an EC_{50} value of 0.138 2 mg/L. Furthermore, the field efficacy trials revealed that 50% fluazinam SC and 62% cyprodinil WG demonstrated superior effectiveness against brown spot, achieving control efficacies of 70.44% and 63.09%, respectively. Sun Chuntai[18] conducted an investigation into the efficacy of eight chemical agents for the management of tobacco brown spot. The study revealed that 40% dimethachlon, 25% prochloraz, 25.5% propiconazole, and 80% chlorothalonil (all administered at a concentration of 10 mg/L) exhibited superior control over tobacco brown spot, with average disease control effects exceeding 74%.

3.3 Biological control While chemical control methods are effective, they can easily result in environmental pollution and pesticide residues, which contradict the principles of sustainable practices in green and organic tobacco cultivation. Furthermore, these methods may contribute to the development of resistance among pathogens. Chen Changqing *et al.*[28] identified that the pathogen responsible for tobacco brown spot in Heilongjiang and Jilin provinces exhibited varying levels of resistance to dimethachlon and difenoconazole. Consequently, biological control has been consistently highlighted as the safest and most effective management strategy. Currently, the biological control measures for tobacco brown spot primarily encompass the use of biocontrol strains, plant extracts, and transgenic breeding techniques.

A significant number of biocontrol strains exhibiting inhibitory effects on tobacco brown spot have been identified. Fang Dunhuang *et al.*[29] isolated a strain of *Bacillus subtilis* B75 from the rhizosphere of healthy tobacco plants. The *in vitro* control efficacy of the fermentation broth containing this bacterium was found to be 76.9%, while the metabolic crude extracts demonstrated a control efficacy of 64.7%. In field trials, the control effects were recorded at 75.8% and 64.4%, respectively. Furthermore, the metabolic crude extract was shown to inhibit spore germination, mycelial growth, and spore production of the pathogen at certain concentrations. Wan Ke[30] isolated a strain of highly effective antagonistic biocontrol bacterium L12. This strain was subsequently identified as *Bacillus megaterium* through a combination of morphological, physiological, biochemical, and molecular identification techniques. The bacterium has been shown to enhance the activities of phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) *in vivo*, thereby contributing to the induction of plant resistance. Chen Xue *et al.*[31] collected 131 samples of both normal and diseased tobacco rhizosphere soils from eight counties in Bijie, Guizhou. From these samples, they identified one strain, designated as L3, which exhibited a significant inhibitory effect on the pathogen responsible for brown spot. This strain was subsequently identified as *B. subtilis* and was found to induce aberrations, breakage, apical expansion, and protoplasmic coalescence in mycelia. Luo Chuxiang *et al.*[32] screened three strains of antagonistic *Bacillus* associated with tobacco brown spot through a combination of plate co-culture primary screening and re-screening using fermentation supernatant filtrate. Among the strains evaluated, the K_{11} strain exhibited the highest percentage inhibition of radial growth (PIRG) at 63.64%. Furthermore, the PIRG value of the fermentation supernatant filtrate reached 67.44%, leading to its identification as *B. subtilis*. Song Lisha *et al.*[33] screened F_{11} strain from tobacco-growing soil in Weng'an County, Guizhou Province. This strain exhibited a significant antagonistic effect against the pathogen responsible for brown spot. The aseptic fermentation supernatant demonstrated an inhibition zone measuring up to 23.5 mm against the growth of the pathogenic fungus, which was identified as *Bacillus methylotrophicus*, a methylotrophic bacterium. Ma Zhiyuan[34] successfully isolated and screened a *Bacillus* strain M-07, which was subsequently identified as *Bacillus amyloliquefaciens*, from the rhizosphere soils and leaves of tobacco plants collected in four tobacco-growing provinces: Shaanxi, Yunnan, Hubei, and Henan. The application of the crude extract at a concentration of 80 mg/L demonstrated a control efficacy of 86.0% in field conditions. Tao Gang *et al.*[35] conducted a screening of *Trichoderma* strains that exhibited high chitinase production and significant antagonistic activity against the pathogen responsible for tobacco brown spot. This was achieved through the use of confrontation plate antagonism assays, colloidal chitin plate transparent circle assays, and the DNS method. Wang Ge *et al.*[36] isolated 18 strains of *Trichoderma* from soil and identified one strain, designated Tv-1, which exhibited a notably strong antagonistic effect against the pathogen responsible for tobacco brown spot, utilizing the confrontation method. The antagonistic mechanisms of strain Tv-1 primarily involved growth competition, parasitism, and the production of antimicrobial compounds that disrupt the mycelium of the pathogen. Yi Long *et al.*[37] isolated 302 strains of non-pathogenic endophytes from the stems and leaves of healthy tobacco plants. Among these, the endophyte strain Itb162 demonstrated a 52.0% efficacy in controlling tobacco brown spot. Furthermore, its sterile filtrate was found to inhibit both mycelial growth and spore germination of the pathogen within a specific concentration range. Qin Baofu *et al.*[38] successfully isolated an endophytic fungus resistance to penicillin from the leaves of *Hyacinthus orientalis*. The crude extract derived from its fermentation broth demonstrated a relative inhibition rate of 93.8% against tobacco brown spot.

The inhibitory effects of plant extracts on pathogens have increasingly garnered attention in recent research. Yuan Yuan[39] reported that 41 ultrasonic extracts derived from Chinese herbal medicines exhibited inhibitory effects against tobacco brown spot. Notably, ten extracts, specifically from *Leonurus artemisia*, *Angelica dahurica*, *realgar*, *Alpinia officinarum*, *Syringa oblata*, *Forsythia suspensa*, *Reynoutria japonica*, *Cinnamomum cassia*, *Zanthoxylum bungeanum*, and *Rheum officinale*, demonstrated inhibition rates up to 100% against this pathogen. Additionally, the extracts from nine other plants, including *Arisaema heterophyllum*, *Coptis chinensis*, *Xanthium sibiricum*, Dioscoreae Bulbiferae Rhizoma, Armeniacae Semen Amarum, *Ginkgo biloba*, and *Asarum sieboldii*, exhibited inhibition rates exceeding 90%. Wang Shaonan *et al.*[40] employed various methods to extract crude extracts from 12 different plant species. Their findings indicated that the water vapor extracts of *Litsea cubeba* fruits, the petroleum ether extracts of *Arctium lappa* seeds, and the water vapor extracts of *Syzygium aromaticum* flowers exhibited a significant inhibitory effect on the spore germination of tobacco brown spot. Furthermore, these extracts demonstrated relatively effective field control, with their efficacy approaching that of the control agent dimethachlon. Zhang Yanzhen *et al.* [41] investigated the inhibitory effects of 95% ethanol crude extracts from *Alpinia oblongifolia*, Fructus Terminaliae Belliricae, *Neolamarckia cadamba*, and *Acmella paniculata* on the fungal pathogen *A. alternata*. Utilizing spore germination and growth rate assays at a final concentration of 200 μg/mL, the study found that *A. oblongifolia* exhibited the highest inhibitory rates, achieving 72% inhibition of spore germination and 53% inhibition of mycelial growth. When compared to the positive control, which was 75% chlorothalonil WP at the same concentration, no statistically significant difference in inhibition rates was observed ($\alpha = 0.05$). Wang Jing *et al.* [42] examined the inhibitory effects of extracts from *Pueraria peduncularis* on the mycelium of five plant pathogens, including *A. alternata*. The results indicated that the inhibition rate against *A. alternata* reached $(82.15 \pm 0.64)\%$.

Transgenic technology offers an innovative strategy for the management of tobacco brown spot. Fang Yuda *et al.* [43] successfully incorporated the rice chitinase gene into the tobacco variety K358 through the mediation of *Agrobacterium tumefaciens*. The progeny of the transgenic tobacco plants expressing the rice chitinase gene demonstrated resistance to the pathogen *A. alternata*. Wang Xiaowen *et al.* [44] introduced the *Lj-L* gene, which encodes non-specific lipid transfer proteins (nsLTPs) that function as antimicrobial agents in *Leonurus artemisia*, and the corresponding transformed gene *Lj-S* into tobacco (*Nicotiana tabacum* cv. Xanthi) using the *A. tumefaciens*-mediated transformation method. This approach resulted in the generation of stable genetically modified transgenic tobacco plants. The disease indices of the transgenic plants containing the *Lj-S* and *Lj-L* genes were recorded at 40.77% and 27.66%, respectively, while the average disease index for untransformed plants reached 100%. Notably, the disease resistance observed in the transgenic *Lj-L* plants was marginally superior to that of the transgenic *Lj-S* plants, but this difference did not achieve statistical significance. Dou Daolong *et al.* [45] successfully cloned the *NDR*1 gene from the *Arabidopsis thaliana* Wassilewskija ecotype utilizing polymerase chain reaction (PCR) techniques. Subsequently, they generated transgenic tobacco plants through *A. tumefaciens*-mediated transformation of the NC89 tobacco variety. From a sample of 10 randomly selected transgenic tobacco plants, two individuals exhibiting resistance to tobacco brown spot were identified. Lan Haiyan *et al.* [46] employed an *A. tumefaciens*-mediated method to introduce the bivalent plant expression vector pBLGC, which was constructed from the tobacco alkaline β-1,3-glucanase and chitinase genes, into the tobacco variety K326. The resulting transgenic tobacco exhibited successful integration of the exogenous genes. *In vivo* inoculation experiments indicated that these transgenic tobacco plants displayed a significant resistance to tobacco brown spot.

3.4 Prediction and forecasting Accurate prediction and forecasting of disease occurrences can facilitate the rational application of chemicals, thereby maximizing their efficacy. This approach not only minimizes the quantity of chemicals utilized but also reduces pesticide residues, all while achieving the desired outcomes in disease prevention and control. Tang Ruoyun *et al.* [47] conducted an analysis of the occurrence and development of tobacco brown spot over 16 years across five points in key tobacco-growing regions of Hunan. Their findings indicated that the seasonal prevalence of tobacco brown spot adhered to a logistic curve, with the incidence degree exhibiting annual fluctuations. Factors such as reduced precipitation, delayed transplanting, increased precipitation during the peak period, and low temperatures in the preceding year were identified as conducive to the onset of the disease. They proposed utilizing occurrence type and cumulative temperature methods to predict the timing of tobacco brown spot outbreaks, as well as employing spore capture and multiple correlation techniques to assess the severity of the disease. Liu Xuemin *et al.* [48] conducted a two-year field survey on tobacco brown spot in Binxian and Zhaozhou counties of Heilongjiang Province. They analyzed 13 sets of epidemiological data related to the disease, employing various growth models. Their findings indicated that the Logistic model provided a superior fit for the dynamic process of tobacco brown spot in the field. The study identified several critical factors influencing the occurrence and prevalence of tobacco brown spot, including initial disease levels, average daily temperature, average daily relative humidity, daily rainfall, and the number of rainfall days. Furthermore, they established a predictive model for the growth rate of tobacco brown spot in the field through stepwise regression analysis. Pu Yu *et al.* [20] conducted a study in Longyang tobacco-growing region of Baoshan City, revealing that the seasonal epidemiological dynamics of tobacco brown spot exhibited a logistic growth curve. Their findings indicated that the timing of topping significantly influenced the prevalence of tobacco brown spot. Consequently, they proposed that varying topping periods should be regarded as a critical pathogenic factor and incorporated into the prediction and forecasting models for tobacco brown spot.

4 Challenges for research on tobacco brown spot

4.1 Study of pathogenesis and virulence The pathogen *A. alternata* is a hybrid species characterized by significant variability in geographic distribution, morphology, and pathogenicity. It is essential to conduct a comprehensive study of the pathogenic mechanisms, virulence differentiation, and population succession patterns of this pathogen. Such research will provide a foundation for the selection of disease-resistant varieties and the strategic arrangement of these resistant cultivars[48].

4.2 Breeding of disease-resistant varieties The absence of resistant varieties is a primary factor contributing to the occurrence and prevalence of tobacco brown spot in China. China possesses a wealth of tobacco germplasm resources and has successfully identified several germplasm resources that exhibit resistance to tobacco brown spot. However, due to various quality constraints, the majority of the resistant germplasm resources are not suitable for mainstream cultivation. Consequently, it is imperative to fully utilize disease-resistant germplasm resources and develop new varieties that possess both superior quality and disease resistance through techniques such as hybrid breeding.

4.3 Screening of low-toxicity and high-efficiency agents Currently, the prevention and control of tobacco brown spot predominantly depend on chemical methods. Numerous experiments have been conducted to identify effective agents for this purpose. However, the repeated application of individual agents has resulted in increased pathogen resistance and diminished effectiveness. Therefore, it is essential to conduct further screenings for agents that are characterized by low toxicity, low residue, and particularly those that are non-toxic and residue-free. Peng Xiwen *et al.* [7] conducted a screening of the preservative 10% etaconazole, which exhibits a strong inhibitory effect on both *A. longipes* and *A. alternata*, demonstrating advantages in terms of non-toxicity and free of residue. Furthermore, given that *Alternaria* comprises a mixed species with geographic variability, the dominant species may differ across regions. Consequently, the efficacy of the same agent may vary significantly in different geographical contexts.

4.4 Development of biological control methods with more stable efficacy Biological control is an environmentally friendly approach that avoids the introduction of pesticide residues and the development of pathogen resistance. Although the various biocontrol strains currently screened exhibit strong antifungal performance in laboratory settings, their efficacy in field conditions remains inconsistent. This instability can be attributed to factors such as limited adaptability, challenges in colonization, and the use of single strains of biocontrol bacteria. Although plant extracts demonstrate considerable inhibitory effects on tobacco brown spot, they remain primarily within the realm of laboratory research, with limited availability of related commercial agents. Despite the enhanced disease resistance observed in transgenic tobacco, several challenges persist, including gene silencing, gene flow, instability of resistance in transgenic plants, potential ecological threats posed by disease-resistant transgene, abnormal growth and development of transgenic plants, variations in organ morphology due to the presence of transgenes, and a compromise in the overall quality of tobacco.

4.5 Necessity for accurate prediction and forecasting The prediction and forecasting of tobacco brown spot primarily rely on general investigations and analyses, while systematic quantitative research remains scarce. Tobacco brown spot is a typical epidemic disease that can lead to widespread outbreaks within a short timeframe when environmental conditions are favorable. Accurate prediction and forecasting are essential, as they provide a foundational basis for effective prevention and control measures. The occurrence of tobacco brown spot is attributed to the interplay among the host, the pathogen, and environmental conditions. A systematic analysis of the influence of each epidemiological factor on the disease's occurrence is essential for accurately predicting the timing of disease epidemics, the extent of damage, and the optimal period for control measures[47].

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