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苹果轮纹病菌对甲基硫菌灵、克菌丹和代森联的敏感性

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摘 要:【目的】检测我国主要苹果产区田间苹果轮纹病菌对甲基硫菌灵、克菌丹和代森联3种杀菌剂的敏感性现状。【方法】采用菌丝生长速率法,检测了我国6个省份的106株苹果轮纹病菌对3种杀菌剂的敏感性,采用Spearman's秩相关系数法对3种杀菌剂的敏感性分别进行两两相关分析,比较了不同省份苹果轮纹病菌对同一种杀菌剂的敏感性差异。【结果】甲基硫菌灵对苹果轮纹病菌的EC_{50}值介于0.814 5~10.700 8 mg·L^{-1},平均为(3.357 9±0.160 7) mg·L^{-1},敏感性频率分布呈连续性偏正态分布,田间苹果轮纹病菌对甲基硫菌灵仍然较为敏感。克菌丹的EC_{50}值介于1.672 2~38.487 3 mg·L^{-1},呈不连续单峰曲线,有敏感性下降的菌株XY7(采自河南信阳,EC_{50}为38.487 3 mg·L^{-1})出现。剔除该菌株后,剩余105个菌株对克菌丹的敏感性频率分布呈连续偏正态分布,EC_{50}平均值为(10.268 1±0.460 0) mg·L^{-1},可建立田间苹果轮纹病菌对克菌丹的敏感基线。代森联的EC_{50}值介于1.276 3~32.132 4 mg·L^{-1},平均为(15.511 9±0.710 7) mg·L^{-1},敏感性频率分布呈连续性偏正态分布,可建立田间苹果轮纹病菌对代森联的敏感基线。相关性分析结果表明,甲基硫菌灵和代森联、克菌丹和代森联之间的敏感性不存在相关性,但甲基硫菌灵和克菌丹的敏感性呈显著正相关。不同省份苹果轮纹病菌对同一种杀菌剂的EC_{50}平均值不存在明显差异($P > 0.05$)。【结论】田间苹果轮纹病菌对甲基硫菌灵、克菌丹和代森联的敏感性水平相对较高,并建立了田间苹果轮纹病菌对克菌丹和代森联的敏感基线。

关键词:苹果轮纹病菌;甲基硫菌灵;克菌丹;代森联;敏感性

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The sensitivity of *Botryosphaeria dothidea* to thiophanate-methyl, captan and metiram

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Abstract:【Objective】*Botryosphaeria dothidea* is widely distributed in most apple growing regions in China, causing ring rot disease of apple. For many years, use of chemical fungicides has been effective for controlling the disease in the areas of apple production. However, the effectiveness of these fungicides has been threatened by the emergence of resistant pathogens in the field. Thiophanate-methyl, captan and metiram have been widely used for controlling the disease in China for many years. So far there has been no report on the sensitivity of *B. dothidea* to these fungicides in China. The objective of this study was to detect the sensitivity level of *B. dothidea* to thiophanate-methyl, captan and metiram using 106 field isolates collected from Shandong, Hebei, Henan, Liaoning, Shanxi and Shaanxi, respectively.【Methods】The sensitivity of *B. dothidea* to fungicides was assessed based on inhibition of mycelial growth by PDA containing serial concentrations of fungicides. Technical grade thiophanate-methyl (a.i. 95.0%), captan (a.i.

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95.0%), and metiram (a.i. 87.0%) were dissolved in 100% acetone, respectively, being adjusted to a concentration of 10 000 mg·L^{-1} stock solution. The stock solution was diluted with 0.1% (ω) polysorbate 80 in water to eight concentrations: 5.0, 10.0, 20.0, 40.0, 80.0, 160.0, 320.0 and 640.0 mg·L^{-1}, then added to the PDA medium to produce eight standard concentrations: 0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 64.0 mg of each fungicide per liter of the medium. A 7-mm-diameter agar disk taken from the margin of a 4-day-old *B. dothidea* colony were placed at the center surface of a series of petri dishes (9 cm diameter) containing PDA amended with various concentrations of fungicides. Four replicate plates were used for each concentration. Mycelial growth was quantified after 4 days of incubation at 26 ℃ in darkness by measuring two perpendicular diameters of the growth ring in each plate, and taking the average (the diameter of the original plug subtracted). The effective concentration EC_{50} (concentration which reduced mycelial growth by 50%) was calculated by regressing the radial growth values (100% control) against the log10 values of the fungicide concentrations using SPSS 16.0 software. The cumulative frequency distribution of EC_{50} value was produced and calculated by using SPSS 16.0 software. The bin width of the histogram was calculated by Scott's rule and the normal distribution of the EC_{50} values of the 106 isolates was analyzed using the Shapiro-wilk test. Cross-resistance patterns were analyzed by lgEC_{50} values using the Spearman rank correlation and a line regression analysis. The differences of the sensitivities among *B. dothidea* isolates collected from different provinces were statistically analyzed using a one-way analysis of the variance (ANOVA) with the SPSS 16.0 software, and means were separated using the Student Newman Keuls (SNK) test. 【Results】The EC_{50} values of 106 *B. dothidea* field isolates collected from different provinces of China to thiophanate-methyl varied from 0.814 5 to 10.700 8 mg·L^{-1}, with an average of (3.357 9±0.160 7) mg·L^{-1}. The frequency distribution of EC_{50} values of 106 isolates to thiophanate-methyl was continuous, unimodal and positively skewed according to the Shapiro-wilk test results. The sensitivities of *B. dothidea* field isolates to thiophanate-methyl were still in high levels. The EC_{50} values to captan were from 1.672 2 to 38.487 3 mg·L^{-1}, with discontinuous frequency distribution curve. Isolate XY7 (Henan province) had the highest EC_{50} values to captan (38.487 3 mg·L^{-1}) among the 106 isolates. However, the frequency distribution of EC_{50} values of the other 105 isolates to captan was continuous, unimodal and positively skewed according to the Shapiro-wilk test results. The mean EC_{50} values of the other 105 isolates were (10.268 1±0.460 0) mg·L^{-1}, which could be used as the sensitivity baselines of *B. dothidea* to captan. The EC_{50} values of 106 *B. dothidea* field isolates to metiram were from 1.276 3 to 32.132 4 mg·L^{-1}, with an average of (15.511 9±0.710 7) mg·L^{-1}. The frequency distribution of EC_{50} values of the 106 isolates to metiram was continuous, unimodal and positively skewed according to the Shapiro-wilk test results. Therefore, the sensitivity baseline of *B. dothidea* to metiram could be established in this study. Spearman rank correlation analysis by lgEC_{50} values indicated that there were positively significant correlations between sensitivities of *B. dothidea* isolates to thiophanate-methyl and captan (r=0.281 3, P=0.003 5), but there were no significant correlations between sensitivities of *B. dothidea* isolates to thiophanate-methyl and metiram (r=0.179 7, P=0.065 3), captan and metiram (r=0.081 1, P=0.408 6), respectively. There were no significant differences among the sensitivities of 106 *B. dothidea* field isolates to each fungicide using the SNK test at P=0.05 level ($P > 0.05$).【Conclusion】The 106 *B. dothidea* field isolates showed a relatively high sensitive level to thiophanate-methyl, captan, and metiram, respectively. The sensitivity baselines of *B. dothidea* to captan and metiram could be established according to this study.

Key words: *Botryosphaeria dothidea*; Thiophanate-methyl; Captan; Metiram; Sensitivity

苹果轮纹病(*Botryosphaeria dothidea*)是苹果生产中的一种主要病害,分布于包含中国在内的许多亚洲地区[1]。该病不仅在生长期发病,危害果实和枝干,还可在果实贮藏期发病,造成烂果,严重影响苹果生产[2-3]。随着'富士'等敏感品种在我国的大量推广种植,该病害已上升为我国山东、辽宁、河南、河北、山西和陕西等苹果产区苹果生产中的主要病害,且逐年加重,并造成巨大的经济损失[4-7]。

长期以来,采用化学防治一直是生产中苹果轮纹病防治的主要措施。我国目前生产中常用的杀菌剂主要有多菌灵(carbendazim)、代森锰锌 (mancozeb)、代森联(metiram)、克菌丹(captan)、甲基硫菌灵(thiophanate-methyl) 和戊唑醇(tebuconazole)等单剂及其复配制剂[8-11]。但是,长期使用化学杀菌剂导致的病原菌的抗药性问题也日益严重[4,12-14]。研究表明,田间苹果轮纹病菌已对多菌灵产生了不同程度的抗性[11,14]。尽管有研究表明,戊唑醇对苹果轮纹病的田间防治效果较好,田间苹果轮纹病菌对戊唑醇相对敏感,但苹果轮纹病菌对戊唑醇抗性风险较高[4,10,15-16],并在山东省果园中发现了已对戊唑醇具有低水平抗性的苹果轮纹病菌[13]。最近研究也表明,田间苹果轮纹病菌已经出现了对同属三唑类杀菌剂苯醚甲环唑和氟硅唑敏感性降低的亚群体,这些菌株与戊唑醇之间具有明显的交互抗性[12]。

甲基硫菌灵、克菌丹和代森联3种杀菌剂在田间苹果轮纹病的防治中已经有很长时间的用药历史,同时也是目前我国生产上仍然在大量使用的常用药剂。截止目前,尽管已有苹果轮纹病菌对甲基硫菌灵敏感性的报道,但该研究所用菌株数较少(22株),时间较早(2002年),无法准确反映目前的整体情况[11];而有关田间苹果轮纹病菌对克菌丹和代森联的敏感性则尚无相关报道。为此,笔者采用菌丝生长速率法,检测了我国主要苹果产区的106个苹果轮纹病菌对甲基硫菌灵、克菌丹和代森联的敏感性现状,分析了供试菌株对3种杀菌剂敏感性间的相关性,并比较了不同省份苹果轮纹病菌对杀菌剂的敏感性差异,以期为苹果轮纹病防治的药剂选择和合理使用提供依据,并为田间苹果轮纹病菌的抗药性检测和治理提供基础。

1 材料和方法

1.1 材料

1.1.1 供试菌株 供试苹果轮纹病菌采集并分离自我国的山东省(59株)、河北省(20株)、辽宁省(11株)、河南省(7株)、山西省(5株)和陕西省(4株),总共106株苹果轮纹病菌(菌株来源信息见表1)。

1.1.2 供试药剂 87.0%(metiram)代森联原药,江苏省南通宝叶化工有限公司产品;95.0%甲基硫菌灵原药 (thiophanate-methyl) 和95.0%克菌丹原药(captan),均为山东潍坊润丰化工股份有限公司产品。

用万分之一电子天平分别准确称取95.0%甲基硫菌灵、95.0%克菌丹和87.0%代森联原药各1.052 6 g、1.052 6 g和1.149 4 g,然后分别溶于丙酮中,配成10 000 mg·L^{-1}的母液,备用。

1.2 方法

1.2.1 药液配置 在预试验的基础上,将代森联、甲基硫菌灵和克菌丹按有效成分含量分别设定0.5、1.0、2.0、4.0、8.0、16.0、32.0和64.0 mg·L^{-1} 8个系列质量浓度处理。用含有0.1%的吐温80的水溶液将杀菌剂母液分别稀释至上述设计浓度的10倍浓度(即5.0、10.0、20.0、40.0、80.0、160.0、320.0 和 640.0 mg·L^{-1}),备用。

1.2.2 含药PDA培养基的制备 在超净工作台中,无菌条件下,将预先融化、灭菌的90 mL马铃薯葡萄糖琼脂(PDA)培养基加入无菌锥形瓶中,并冷却至45 ℃,然后用移液枪分别加入1.2.1中配好的各处理药液10 mL,充分摇匀后等量倒入直径为9 cm的培养皿中,制成相应设计浓度的含药PDA平板。以不含药剂的0.1%的吐温80水溶液处理作空白对照。

1.2.3 苹果轮纹病菌对3种杀菌剂的敏感性测定 先将苹果轮纹病菌在PDA培养基上活化4 d,然后在超净工作台内,无菌条件下,用内径为7.0 mm的灭菌打孔器从菌落边缘打取菌柄,并用接种器将菌饼接种于1.2.2中制备好的含药平板中央,菌丝面朝下,盖上皿盖,放入26 ℃恒温培养箱内黑暗培养。每处理4个重复,每个重复1皿。

连续培养4 d后,用直尺采用十字交叉法垂直测量各处理菌落直径各1次,求其平均值,减去7.0 mm菌饼直径,即为菌落增长直径。测量每个重复试验结果,然后求出每个处理浓度的菌落增长直径平均值,并计算每个处理对病原菌的菌丝增长抑制率。

菌丝生长抑制率/%=[(对照菌落生长直径-处理菌落生长直径)/对照菌落生长直径]×100

1.2.4 数据统计与分析 采用SPSS16.0统计分析

表1 田间苹果轮纹病菌菌株来源分布

Table 1 Places and distribution of *Botryosphaeria dothidea* isolates from different provinces in China

菌株 Isolates	省份(个数) Provinces (Number)	地点 Location
BD1	河北(20)	河北保定 Baoding, Hebei
BD15	Hebei (20)	河北保定 Baoding, Hebei
BD2		河北保定 Baoding, Hebei
BD3		河北保定 Baoding, Hebei
CL12		河北昌黎 Changli, Hebei
CL14		河北昌黎 Changli, Hebei
CL16		河北昌黎 Changli, Hebei
CL17		河北昌黎 Changli, Hebei
CL18		河北昌黎 Changli, Hebei
CL19		河北昌黎 Changli, Hebei
FN2		河北抚宁 Funingi, Hebei
FN3		河北抚宁 Funingi, Hebei
FN4		河北抚宁 Funingi, Hebei
FN8		河北抚宁 Funingi, Hebei
QL19		河北青龙 Qinglong, Hebei
QL20		河北青龙 Qinglong, Hebei
QL21		河北青龙 Qinglong, Hebei
QL24		河北青龙 Qinglong, Hebei
QL28		河北青龙 Qinglong, Hebei
QL32		河北青龙 Qinglong, Hebei
MC	河南(7)	河南渑池 Mianchi, Henan
MC10	Henan (7)	河南渑池 Mianchi, Henan
MC6		河南渑池 Mianchi, Henan
103XY7		河南信阳 Xinyang, Henan
YX3		河南信阳 Xinyang, Henan
YX5		河南信阳 Xinyang, Henan
YX7		河南信阳 Xinyang, Henan
HLD10	辽宁(11)	辽宁葫芦岛 Huludao, Liaoning
HLD11	Liaoning (11)	辽宁葫芦岛 Huludao, Liaoning
HLD14		辽宁葫芦岛 Huludao, Liaoning
HLD17		辽宁葫芦岛 Huludao, Liaoning
HLD18		辽宁葫芦岛 Huludao, Liaoning
SZ3		辽宁绥中 Suizhong, Liaoning
SZ5		辽宁绥中 Suizhong, Liaoning
XC21		辽宁兴城 Xingcheng, Liaoning
XC22		辽宁兴城 Xingcheng, Liaoning
XC27		辽宁兴城 Xingcheng, Liaoning
XC49		辽宁兴城 Xingcheng, Liaoning
1DE2	山东(59)	山东东阿 DongE, Shandong
1DE6	Shandong (59)	山东东阿 DongE, Shandong
2DE1		山东东阿 DongE, Shandong
DE		山东东阿 DongE, Shandong
DE2		山东东阿 DongE, Shandong
DE21		山东东阿 DongE, Shandong
DE3		山东东阿 DongE, Shandong
DE6		山东东阿 DongE, Shandong
HM1		山东惠民 Huimin, Shandong
HM18		山东惠民 Huimin, Shandong
HM2		山东惠民 Huimin, Shandong
HM22		山东惠民 Huimin, Shandong
HM24		山东惠民 Huimin, Shandong
HM25		山东惠民 Huimin, Shandong
HM26		山东惠民 Huimin, Shandong
HM27		山东惠民 Huimin, Shandong
HM3		山东惠民 Huimin, Shandong
HM4		山东惠民 Huimin, Shandong
HM5		山东惠民 Huimin, Shandong
HM6		山东惠民 Huimin, Shandong
HM7		山东惠民 Huimin, Shandong
HM8		山东惠民 Huimin, Shandong
HM9		山东惠民 Huimin,Shandong
LK1		山东龙口 Longkou, Shandong
LK2		山东龙口 Longkou, Shandong
LK3		山东龙口 Longkou, Shandong
LK4		山东龙口 Longkou, Shandong
LK41		山东龙口 Longkou, Shandong
LK42		山东龙口 Longkou, Shandong
LK5		山东龙口 Longkou, Shandong
LK51		山东龙口 Longkou, Shandong
LK52		山东龙口 Longkou, Shandong
LK6		山东龙口 Longkou, Shandong
LK61		山东龙口 Longkou, Shandong
LK7		山东龙口 Longkou, Shandong
LK9		山东龙口 Longkou, Shandong
LK10		山东龙口 Longkou, Shandong
LK11		山东龙口 Longkou, Shandong
LK12		山东龙口 Longkou, Shandong
LK13		山东龙口 Longkou, Shandong
LK14		山东龙口 Longkou, Shandong
LK15		山东龙口 Longkou, Shandong
MY5		山东蒙阴 Mengyin, Shandong
MY7		山东蒙阴 Mengyin, Shandong
MY8		山东蒙阴 Mengyin, Shandong
MY9		山东蒙阴 Mengyin, Shandong
PL3		山东蓬莱 Penglai, Shandong
PL4		山东蓬莱 Penglai, Shandong
PL5		山东蓬莱 Penglai, Shandong
210PY1		山东平阴 Pingyin, Shandong
210PY2		山东平阴 Pingyin, Shandong
212PY5		山东平阴 Pingyin, Shandong
PY1		山东平阴 Pingyin, Shandong
QX11		山东栖霞 Qixia, Shandong
QX12		山东栖霞 Qixia, Shandong
QX13		山东栖霞 Qixia, Shandong
TS15		山东泰山 Taishan, Shandong
TS16		山东泰山 Taishan, Shandong
TS7ck		山东泰山 Taishan, Shandong
SXDL18	山西(5)	山西大梁 Daliang, Shanxi
SXDL4	Shanxi (5)	山西大梁 Daliang, Shanxi
SXWRRT15		山西万荣 Wanrong, Shanxi
SXWRRT25		山西万荣 Wanrong, Shanxi
SXWRRT31		山西万荣 Wanrong, Shanxi
SSXDL25	陕西(4)	陕西大荔 Dali, Shaanxi
SSXDL4	Shaanxi (4)	陕西大荔 Dali, Shaanxi
YN1		陕西延南 Yannan, Shaanxi
YN32		陕西延南 Yannan, Shaanxi

软件,以药剂浓度对数值为横坐标,菌丝增长抑制率概率值为纵坐标,进行回归分析,求出杀菌剂对病原菌的EC_{50}、相关系数、回归方程以及95%置信限;采用Shapiro-Wilk检验方法,就供试菌株对杀菌剂的敏感性频率分布进行正态性检验;将EC_{50}值转换为$\lg EC_{50}$值,采用Spearman's秩相关系数就苹果轮纹病菌对3种杀菌剂的敏感性分别进行两两相关性分析[4],并采用SNK检验法比较不同省份苹果轮纹病菌对3种杀菌剂的敏感性差异。以EC_{50}值为横坐标,菌株分布频数为纵坐标,采用Microsoft Excel 2010绘制频数分布柱状图。柱形图的分类间隔值(单根柱的宽度)采用Scott[17]的公式计算:$H=3.49SN^{-1/3}$,其中H为分类间隔值(binwidth);S为EC_{50}值的标准偏差(standard deviation);N为测定的菌株数量。然后依据EC_{50}值分布范围确定柱体数量[18]。

2 结果与分析

2.1 苹果轮纹病菌对3种杀菌剂的敏感性检测

甲基硫菌灵对苹果轮纹病菌的EC_{50}值介于0.814 5~10.700 8 mg·L^{-1}，EC_{50}平均值为(3.357 9±0.160 7) mg·L^{-1}，最高值为最低值的13.14倍(表2)。敏感性频率分布的正态性检验结果表明，当显著水平为95%时，偏度skew=2.020，峰度kurt=5.796，W=0.828，$P<$ 0.000 1，符合连续的偏正态分布，且所测106株菌株对甲基硫菌灵的敏感性频率分布为连续单峰曲线(图1)。表明不同来源的田间苹果轮纹病菌对甲基硫菌灵的敏感水平相对较高，未出现敏感性降低的亚群体。

克菌丹的EC_{50}值介于1.672 2~38.487 3 mg·L^{-1}，EC_{50}平均值为(10.534 3±0.527 7) mg·L^{-1}，最高值为最低值的23.02倍(表2)。但敏感性频率分布呈不连续单峰曲线，表明田间苹果轮纹病菌对克菌丹的敏感性出现了分化，有敏感性下降的菌株出现。将敏感性最低的菌株XY7(采自河南信阳，EC_{50}为38.487 3 mg·L^{-1})剔除后，剩余105株苹果轮纹病菌对克菌丹的敏感性频率分布呈连续单峰曲线(图2)，EC_{50}平均值为(10.268 1±0.460 0) mg·L^{-1}。进一

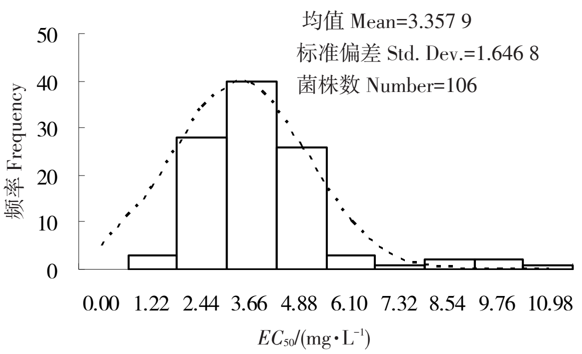
表2 不同省份苹果轮纹病菌对甲基硫菌灵、克菌丹和代森联的敏感性水平

Table 2 The sensitivity level of *B. dothidea* isolated from different provinces in China to thiophanate-methyl, captan and metiram

杀菌剂 Fungicides	省份(数目) Provinces(numbers)	EC_{50}范围 Range of EC_{50}/(mg·L^{-1})	EC_{50}均值±标准误 Mean±SE	变异系数 *CV*
甲基硫菌灵 Thiophanate-methyl	山东Shandong(59)	0.814 5~10.700 8	3.480 5±0.250 6 a	0.552 9
	河北 Hebei(20)	1.056 4~5.774 1	3.127 4±0.245 9 a	0.351 6
	辽宁Liaoning(11)	1.658 5~4.402 4	3.131 1±0.239 6 a	0.253 8
	河南Henan(7)	1.271 1~8.128 0	3.591 1±0.842 7 a	0.620 8
	山西Shanxi(5)	1.514 3~4.773 7	3.305 6±0.627 3 a	0.424 3
	陕西Shaanxi(4)	1.763 4~3.681 8	2.983 1±0.427 4 a	0.286 6
	总计Total (106)	0.814 5~10.700 8	3.357 9±0.160 7	0.492 7
克菌丹 Captan	山东Shandong(59)	2.865 4~27.009 5	10.320 5±0.632 6 a	0.470 8
	河北 Hebei(20)	1.672 2~18.454 9	11.103 9±1.059 0 a	0.426 5
	辽宁Liaoning(11)	3.101 3~15.173 6	8.260 3±1.157 7 a	0.464 8
	河南Henan(7)	6.457 4~38.487 3	14.921 3±4.340 2 a	0.769 6
	山西Shanxi(5)	3.411 2~15.726 1	9.947 0±2.057 1 a	0.462 4
	陕西Shaanxi(4)	4.764 1~17.015 1	10.150 3±2.544 8 a	0.501 4
	总计Total (106)	1.672 2~38.487 3	10.534 3±0.527 7	0.518 8
代森联 Metiram	山东Shandong(59)	1.276 3~32.132 4	15.090 1±0.938 7 a	0.477 8
	河北 Hebei(20)	3.290 5~23.259 8	14.673 4±1.491 4 a	0.454 5
	辽宁Liaoning(11)	1.327 7~31.248 8	17.042 6±2.596 1 a	0.505 2
	河南Henan(7)	2.396 3~23.477 3	13.926 9±3.139 0 a	0.596 3
	山西Shanxi(5)	4.024 7~27.408 7	17.667 6±4.032 8 a	0.510 4
	陕西Shaanxi(4)	17.730 8~25.519 6	21.795 9±1.602 9 a	0.147 1
	总计Total (106)	1.276 3~32.132 4	15.511 9±0.710 7	0.471 7

注：同列数据后的小写字母表示由SNK检验后在P=0.05水平上的差异显著性。

Note: The small letters within a row indicate a difference at at P=0.05 level using the Student Newman Keuls (SNK) test.

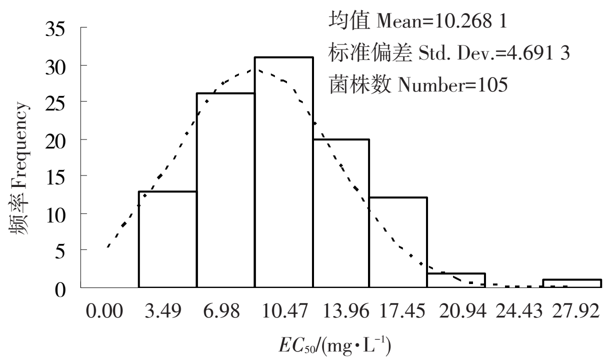


柱宽度(1.22)是按照Scott的公式计算获得。

The bin width of 1.22 was calculated by Scott's rule.

图1 苹果轮纹病菌对甲基硫菌灵敏感性的频率分布

Fig. 1 The frequency distribution of sensitivity of 106 *B. dothidea* isolates to thiophanate-methyl by EC_{50} values



柱宽度(3.49)是按照Scott的公式计算获得。

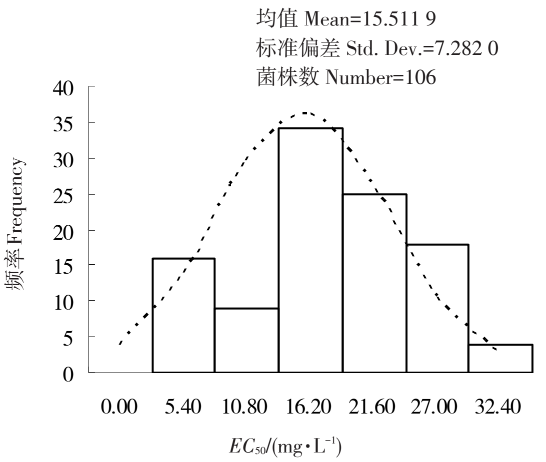
The bin width of 3.49 was calculated by Scott's rule.

图2 苹果轮纹病菌对克菌丹敏感性的频率分布

Fig. 2 The frequency distribution of sensitivity of 105 *B. dothidea* isolates to captan by EC_{50} values

步的正态性检验结果表明，当显著水平为95%时，偏度skew=0.611，峰度kurt =0.518，W=0.973，P=0.028<0.05，符合连续的偏正态分布。因此，可以采用EC_{50}平均值10.268 1±0.460 0 mg·L^{-1}作为田间苹果轮纹病菌菌丝生长对克菌丹的敏感基线。

代森联的EC_{50}值介于1.276 3~32.132 4 mg·L^{-1}，EC_{50}平均值为(15.511 9±0.710 7) mg·L^{-1}，最高值为最低值的25.36倍（表2）。正态性检验结果表明，当显著水平为95%时，偏度skew=−0.223，峰度kurt=−0.594，W=0.967，P=0.009<0.05，符合连续的偏正态分布，且所测106株菌株对代森联的敏感性频率分布为连续单峰曲线（图3）。因此，可以采用EC_{50}平均值(15.511 9±0.710 7) mg·L^{-1}作为田间苹果轮纹病菌菌丝生长对代森联的敏感基线。



柱宽度(5.40)是按照Scott的公式计算获得。
The bin width of 5.40 was calculated by Scott's rule.

图3　苹果轮纹病菌对代森联敏感性的频率分布

Fig. 3 The frequency distribution of sensitivity of 106 *B. dothidea* isolates to metiram by EC_{50} values

2.2　苹果轮纹病菌对3种杀菌剂敏感性的相关性分析

苹果轮纹病菌对甲基硫菌灵和克菌丹敏感性的相关分析结果表明（图4），相关系数(r)为0.281 3 (P = 0.003 5 < 0.01)，表明这2种药剂之间存在明显的正相关性。苹果轮纹病菌对甲基硫菌灵和代森联的敏感性之间的相关系数(r)为0.179 7(P=0.065 3 > 0.05)，表明这2种药剂之间不存在相关性（图5）。苹果轮纹病菌对克菌丹与代森联的敏感性之间的相关系数(r)为0.081 1(P=0.408 6 > 0.05)，表明克菌丹和代森联之间也不存在明显的相关性（图6）。

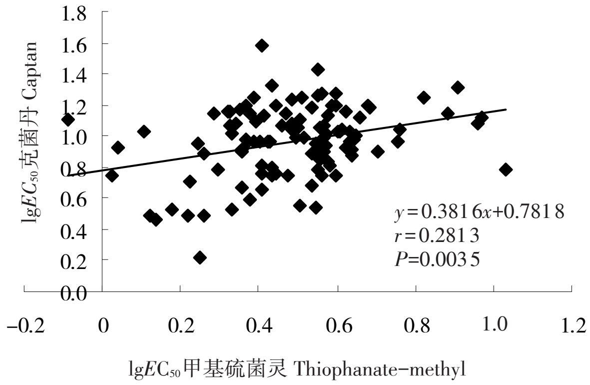


图4　苹果轮纹病菌对甲基硫菌灵和克菌丹的交互抗性

Fig. 4 Cross-resistance of *B. dothidea* to thiophanate-methyl and captan by lgEC_{50} values

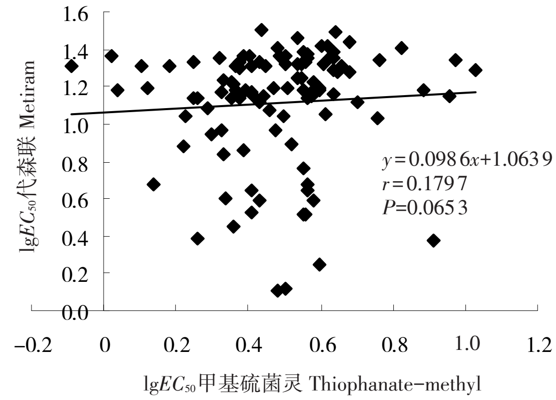


图5　苹果轮纹病菌对甲基硫菌灵和代森联的交互抗性

Fig. 5 Cross-resistance of *B. dothidea* to thiophanate-methyl and metiram by lgEC_{50} values

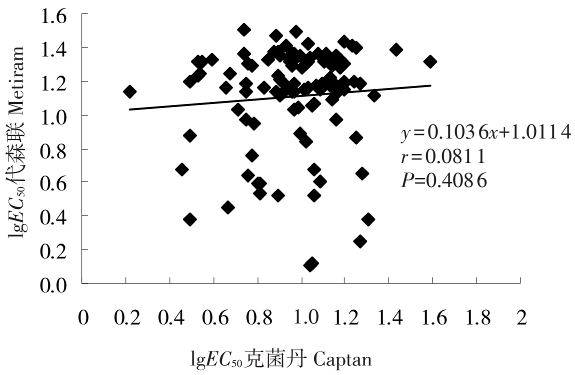


图6　苹果轮纹病菌对克菌丹和代森联的交互抗性

Fig. 6 Cross-resistance of *B. dothidea* to captan and metiram by lgEC_{50} values

2.3　不同地区苹果轮纹病菌对3种杀菌剂的敏感性水平

采用SNK检验法比较了不同省份苹果轮纹病菌对同一种杀菌剂的敏感性差异，统计分析结果表明（表2），尽管不同省份采集的苹果轮纹病菌对同一种杀菌剂的EC_{50}平均值存在差异，但差异不明显

（$P < 0.05$）。

3 讨 论

甲基硫菌灵主要通过转化为多菌灵对病害起到防治作用，尽管有报道2种药剂之间存在交互抗性[11]。但最近研究发现[19]，供试田间苹果轮纹病菌对甲基硫菌灵均表现较高的敏感性，EC_{50}值介于0.07~0.64 mg·L^{-1}。刘保友等[20]研究也发现，山东省的田间苹果轮纹病菌对甲基硫菌灵的敏感水平较高，未出现敏感性降低的群体，甲基硫菌灵对山东省的76株田间苹果轮纹病菌的EC_{50}平均值为（3.883 6±2.097 9）mg·L^{-1}，可作为敏感基线。本研究检测了我国6个省份的106株苹果轮纹病菌对甲基硫菌灵的敏感性，结果表明，甲基硫菌灵对田间苹果轮纹病菌的EC_{50}平均值为（3.357 9±0.160 7）mg·L^{-1}，其敏感性频率分布呈连续偏正态分布；且笔者测得的59株山东省苹果轮纹病菌的EC_{50}平均值为（3.480 5±0.250 6）mg·L^{-1}，研究结果与刘保友等[20]的研究结果基本吻合。这说明田间苹果轮纹病菌对甲基硫菌灵仍保持较高的敏感性，未出现敏感性降低的亚群体。

克菌丹也是我国长期以来被用于防治苹果轮纹病的重要品种。它是一种广谱的有机硫杀菌剂，具有治疗和保护双重作用，作用位点多，主要通过影响丙酮酸脱羧作用和抑制α-酮戊二酸脱氢酶系活性发挥抑菌作用。张伟等[21]曾测得克菌丹对1株苹果轮纹病菌的EC_{50}值为9. 671 1 mg·L^{-1}。但一直未有田间苹果轮纹病菌对克菌丹敏感性检测的相关报道。本研究检测了克菌丹对106株田间苹果轮纹病菌的敏感性，检测到了1个敏感性下降的菌株XY7（采自河南信阳，EC_{50}为38.487 3 mg·L^{-1}）。剔除该菌株后，剩余105个菌株的EC_{50}值介于1.672 2~27.009 5 mg·L^{-1}，平均为（10.268 1±0.460 0）mg·L^{-1}，与张伟等[21]测得的EC_{50}较为接近。且这105个菌株对克菌丹敏感性的频率分布符合偏正态分布，因此平均值（10.268 1±0.460 0）mg·L^{-1}可以用来作为田间苹果轮纹病菌菌丝生长对克菌丹的敏感基线。

代森联和代森锰锌也是我国生产中经常被用于防治苹果轮纹病的2个乙撑二硫代氨基甲酸盐(EB-DCs)类保护性杀菌剂。由于该类杀菌剂作用位点多，一般来说，病原菌不易对其产生抗性[22]。刘保友等[23]和范昆等[24]分别采用菌丝生长速率法检测了山东省苹果轮纹病菌对代森锰锌的敏感性，均发现山东省苹果轮纹病菌对代森锰锌的敏感性水平相对较高，未发现抗性菌株。张成玲等[25]曾测得代森联对1株苹果轮纹病菌的EC_{50}值为10.650 mg·L^{-1}，但有关田间苹果轮纹病菌对代森联的敏感性检测研究尚未见报道。本研究检测了我国6个省份的106株田间苹果轮纹病菌对代森联的敏感性，结果表明，EC_{50}值介于1.276 3~32.132 4 mg·L^{-1}，平均值为(15.511 9±0.710 7) mg·L^{-1}。且其敏感性频率分布呈连续的偏正态分布，因此其平均值可以作为苹果轮纹病菌对代森联的敏感基线。但并不意味着苹果轮纹病菌不会对代森联和代森锰锌这类多作用位点的保护性杀菌剂产生抗药性，若在田间长期不合理的过度应用，也会导致田间病原菌对其产生抗性[22-23]。

不同地理来源的病原菌对同一种杀菌剂的敏感性会存在明显差异[26]。笔者前期研究发现[27]，不同省份的田间苹果轮纹病菌对腐霉利的敏感性存在明显差异（$P < 0.05$）。但本研究发现，同样的6个省份苹果轮纹病菌对甲基硫菌灵、克菌丹和代森联的平均EC_{50}值不存在明显差异（$P < 0.05$）。已有的研究亦证明，不同地理来源的苹果轮纹病菌对多菌灵和代森锰锌的敏感性差异不明显[24,28]。但由于该研究中河南（7株）、陕西（4株）和山西（5株）3省所用的苹果轮纹病菌相对较少，无法准确判断不同地理来源的苹果轮纹病菌对所测定杀菌剂的敏感性差异以及变异程度。事实上，田间植物病原菌对药剂的敏感性水平差异，除了与病原菌的采集时间和地理来源分布、寄主作物不同种植年限、病害发生程度有一定关系外，当地农民所采用的药剂种类，用药历史、习惯和水平均会导致杀菌剂敏感水平差异的产生[28]。因此，甲基硫菌灵、克菌丹和代森联对我国不同省份苹果轮纹病菌的敏感性差异，尚需增加更多的有代表性的田间菌株，进一步进行研究比较。

田间苹果轮纹病菌对供试3种杀菌剂敏感性间的相关性分析表明，甲基硫菌灵与克菌丹之间存在明显的正相关，而甲基硫菌灵与代森联，克菌丹与代森联之间不存在明显的相关性。其原因可能是甲基硫菌灵和克菌丹及其复配制剂在我国不同省份被同时应用于苹果轮纹病的防治，田间同时存在这2种药剂的选择压力，导致田间苹果病原菌对甲基硫菌灵和克菌丹的敏感性存在明显相关性。

4 结　论

田间苹果轮纹病菌对甲基硫菌灵仍保持较高的敏感性，未出现敏感性降低的亚群体。田间苹果轮纹病菌对克菌丹和代森联的敏感性水平也相对较高，并建立了田间苹果轮纹病菌菌丝生长对克菌丹和代森联的敏感基线；甲基硫菌灵和代森联、克菌丹和代森联之间的敏感性不存在相关性，但甲基硫菌灵和克菌丹的敏感性呈显著正相关。不同省份田间苹果轮纹病菌对同一种杀菌剂的敏感性水平不存在明显差异。

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