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Pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum* in Asia

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Anthracnose of chili is caused by a complex of *Colletotrichum* species, with recent surveys reporting at least 28 different species implicated. However, there have been very few studies to identify the relative pathogenicity of the various species or to optimize a bioassay to assess pathogenicity. A detached *Capsicum* fruit bioassay to determine the pathogenicity of a diverse geographical range of isolates of *Colletotrichum scovillei* showed fruit maturity, host genotype, and inoculation method all interact to affect infection and rate of lesion development. On *Capsicum annuum* 'Bangchang' fruit wounded prior to inoculation, pathogenicity was consistent regardless of fruit maturity. In contrast, without wounding there was variability in pathogenicity. On the relatively resistant host *Capsicum chinense* PBC932, pathogenicity was dependent on both the inoculation method and maturity stage of the fruit. In addition, lack of correlation in pathogenicity of isolates between the two *Capsicum* lines indicated that there was host–isolate specialization that would make prediction of pathogenicity of isolates on host difficult. In a further study, 10 species of *Colletotrichum* isolated from diseased chili fruits in Asia caused anthracnose symptoms on *C. annuum* 'Bangchang' under all testing conditions, with large differences in aggressiveness. *C. chinense* PBC932 was generally more resistant to all the species, with smaller lesions produced in different host conditions. *Colletotrichum javanense* and *C. scovillei* were highly aggressive relative to other species, especially when inoculated on nonwounded fruit. Pathotype differences were identified within multiple isolates of *C. scovillei* and *C. siamense*, the two most frequently identified pathogenic species on chili.

1 Introduction

Most of the species of *Colletotrichum* causing anthracnose of chili are members of the acutatum, gloeosporioides, and truncatum species complexes, but recently species from the other 11 complexes have also been identified (De Silva et al., 2017a; Diao et al., 2017; Liu et al., 2016). While associated with anthracnose of chili, the pathogenicity of these species is not well understood, and some may in fact not be true pathogens of chili. Some may have a latent phase and become pathogenic for only a short part of their life cycle (De Silva et al., 2017b). Others may be specialized to specific hosts and only pathogenic on certain chili lines or particular plant organs, for example on leaves but not fruits.

Most of the reported pathogenicity studies of *Colletotrichum* spp. infecting *Capsicum* have been carried out using a fruit bioassay involving placement of the inoculum (spores or mycelial plugs) over a fresh wound to the epidermis of the fruit (Diao et al,. 2017; Mahasuk et al., 2013; Mongkolporn et al., 2010). In contrast, De Silva et al. (2017b, 2019) found a large difference in host reaction of *Capsicum annuum* fruit depending on whether the fruit had been wounded or nonwounded before inoculation with *Colletotrichum* species originating from diseased chili plants in Asia and Australia. In general, wounding fruit prior to inoculation resulted in infection by all species, whereas nonwounding prevented infection by some species and some isolates within a species. Isolates that are nonpathogenic on nonwounded fruits may be highly damaging when infection occurs through a wound. Fruit maturity is also a major factor affecting pathogenicity of a species or isolate. Mongkolporn et al. (2010) and Prusky et al. (2013) showed that the physiological maturity stage of fruit may affect the aggressiveness of a pathogen.

Pathotypes of *Colletotrichum* species based on qualitative, differential host reactions (i.e., the ability or inability to cause disease in specific host genotypes) have been reported on chili (Diao et al., 2017; Mongkolporn et al., 2010; Montri et al., 2009). Knowledge of variation in aggressiveness of the pathogen and pathotype structure within pathogen populations is important for development of effective control strategies for targeted pathogen species and efficient breeding of resistant host lines. Mongkolporn et al. (2010) identified pathotypes of *C. truncatum*, *C. scovillei* (formerly *C. acutatum*) and *C. siamense* (formerly *C. gloeosporioides*) within populations of each species in Thailand. Pathotypes were identified on infected chili fruits at two maturity stages (red, mature green) of 10 genotypes from four *Capsicum* spp.—*C. annuum*, *C. baccatum*, *C. chinense*, and *C. frutescens*—with wound inoculation.

C. annuum is the most important commercial *Capsicum* species worldwide but cultivars lack resistance to anthracnose. However, anthracnose resistance, identified in a specific inbred resistant line (fixed genotype PBC932) of *C. chinense* by the World Vegetable Center in Taiwan (Mongkolporn & Taylor, 2011), was successfully crossed with *C. annuum* to produce hybrid lines with putative anthracnose resistance genes. Previous studies (DeSilva et al., 2017a, 2017b; Monkolporn et al., 2010; Montri et al., 2009) showed that *C. annuum* 'Bangchang' was very susceptible and *C. chinense* PBC932 was resistant, with the latter genotype producing differential reactions to isolates of *Colletotrichum* spp. Hence, pathotypes that can overcome resistance from *C. chinense* would be of major concern to plant breeders. Thus, these two host lines were selected for optimizing the bioassay and screening for different *Colletotrichum* spp. and isolates.

A previous study on taxonomy and phylogeny of the *Colletotrichum* species causing anthracnose in *Capsicum* spp. in Asia identified 11 species of *Colletotrichum* including three novel species, *C. javanense*, *C. makassarense*, and *C. tainanense* (De Silva et al., 2019). Of these species, *C. truncatum* had already been identified as a major pathogen of chili and its pathogenicity was determined by Diao et al. (2017), Mongkolporn et al. (2010), Montri et al. (2009), Ranathunge et al. (2012), and Tariq et al. (2017). *C. truncatum* produces falcate spores which can be readily identified microscopically. Most of the other *Colletotrichum* spp. associated with chili anthracnose have straight conidia (fusiform and obtuse) and are difficult to differentiate by morphological characters alone. This difficulty in knowing which species are involved in causing disease has meant there have been very few studies on pathogenicity of the range of potentially important pathogens. The objectives of this study were to: (a) investigate the interaction between fruit maturity, host line, and inoculation method (with or without wounding) to develop an efficient, detached-fruit bioassay for pathogenicity of *C. scovillei*; (b) determine the pathogenicity and aggressiveness of 10 *Colletotrichum* species with straight conidia using the detached fruit bioassays; and (c) identify pathotype differences in isolates of *C. scovillei* and *C. siamense*, two of the most frequently identified and pathogenic species on chili.

2 Materials and methods

2.1 Plant material

Chili plants of *C. annuum* 'Bangchang' and *C. chinense* PBC 932 were established in the glasshouse at the University of Melbourne (UM). These two lines were important differential hosts used in previous studies to identify pathotypes of *Colletotrichum* spp. infecting chili fruit (Mongkolporn et al., 2010). Ripe red and mature green chili fruits from both lines were selected for bioassays.

2.2 Isolates and inoculum preparation

Ten *Colletotrichum* species comprising a total of 46 isolates cultured from diseased chili fruits collected in Indonesia, Malaysia, Taiwan, and Thailand were used in the bioassays

(Table 1). Isolates were maintained on potato dextrose agar (PDA) and incubated for 7–10 days at 25 °C until cultures sporulated. Conidia were obtained by gently scraping the surface of the cultures with a sterilized scalpel with 2–3 ml sterile distilled water. Spore suspensions were filtered through muslin and a sterilized funnel. The final concentration of 10^6 spores/ml was obtained by diluting with sterile distilled water and adjusting using a haemocytometer.

2.3 Chili fruit inoculation bioassay

Mature green and red chili fruit were carefully harvested from the plants so as not to bruise the cuticle, rinsed with water, and surface sterilized with 1% (a.i.) sodium hypochlorite for 2 min, rinsed twice with sterile distilled water (SDW), then air-dried in a laminar flow cabinet. Two inoculation techniques, wounding and nonwounding, were applied to the fruit. For the wounding method, the cuticle and periderm of fruits were wounded approximately 2 cm from the peduncle by pricking with a sterile needle to about 1 mm depth, followed by placing 10 µl of conidial suspension over the wound by pipette. Wounding produced an approximately 2 mm deep hole in the fruit. For the nonwounding method, 10 µl of conidial suspension was placed directly on the surface of the intact cuticle, 2 cm from the base of the fruit. Control fruits (both wounded and nonwounded) were inoculated with 10 µl SDW. Inoculated fruit were placed on a sterilized tray in a clean plastic box with SDW at the bottom to keep humidity high. The containers were sealed and maintained in an incubator at 27 °C. Three replicate fruits were tested per isolate for both inoculation methods, and the experiment repeated three times.

Lesion development was measured 14 days after inoculation (DAI) by measuring the longitudinal diameter of each lesion. Disease symptoms for the lesion size measurements were considered as the fruit area showing necrosis or a larger water-soaked region surrounding the inoculation site; lesion size >0 mm was considered as a lesion for nonwounded inoculation and lesion size ≥2 mm for wounded inoculation; 0 = no infection. At the conclusion of the trials, fruit tissue from nonwounded inoculated fruit without observable symptoms were cultured to assess for the presence of the pathogen.

2.4 Data analysis

2.4.1 Detached fruit bioassay for *C. scovillei*

Lesion size was analysed initially by fitting an analysis of variance (ANOVA) model with the four factors (isolate, host line, physiological maturity, and inoculation method) and all their possible interactions to order 3 using the GLM (general linear model) procedure in SAS v. 9.4 software. This analysis showed most of the potential interactions were significant, including isolate × host line × physiological maturity × inoculation method. Complex, multifactor interactions are difficult to interpret, so the data were split into separate host line × physiological maturity × inoculation method subsets, representing a particular pathogenicity "testing environment", and a one-factor ANOVA model for isolates fitted within each. That also avoided the problem of heteroskedastic residuals resulting from scale differences between the testing environments. Least squares means and their 95% confidence intervals were estimated for each testing environment, the results categorized by two of the factors and their consistency between the two levels of the remaining factor were evaluated by Pearson correlations of the isolate means.

2.4.2 *Colletotrichum* species pathogenicity analysis

Data were analysed using Minitab v. 18 by fitting the linear, mixed model, with isolate within host line included as a random effect and line included as a fixed effect. Least squares means were estimated for each species and Fisher pairwise comparisons were carried out between each pair of means at 95% confidence.

Pathotypes were identified between the isolates of the species of *C. scovillei* and *C. siamense* where qualitative differences in lesion development (ability or inability to infect) were observed on a specific chili line at the same fruit maturity stage with same inoculation method.

3 Results

3.1 Detached fruit bioassay for *C. scovillei*

Despite the complexity and statistical significance of the four factor interactions, *C. annuum* 'Bangchang' was more susceptible overall than *C. chinense* PBC932 and lesion size tended to be larger on wounded than nonwounded fruit. There were very highly significant differences ($p < .001$) between isolates under all the assay techniques except for *C. annuum*, red, wounded; while still significant, the probability was larger ($p < .003$) probably because all isolates caused large lesions, so discrimination between them was relatively poor (Figure 1).

Classification by host lines and fruit maturity (Figure 1a) showed that there was a significant positive correlation (r = .76) of lesion size between wounded and nonwounded *C. annuum* green fruit, and a weaker, nonsignificant correlation (r = .37) between wounded and nonwounded red fruit. However, the correlations of lesion size between the two inoculation methods were effectively zero for *C. chinense* at both fruit maturity stages.

Classification of the bioassay by inoculation technique and host lines (Figure 1b) showed a positive and significant correlation of lesion size between red and green fruit for *C. annuum* nonwounded (r =.56) and wounded (r = .37). A very small level of infection occurred, and mean lesion sizes were very small in nonwounded *C. chinense* fruit, with only a few isolates being able to infect and produce lesions in either red or green fruit. The lack of correlation may not be very meaningful given the low absolute magnitude of the variation and the small sample sizes. In contrast, lesion sizes were much larger and more variable when the fruit were wounded, but still the correlation between red and green fruit was almost zero.

Classification by wounding and fruit maturity (Figure 1c) clearly showed that there was no association between lesion sizes on *C. annuum* 'Bangchang' and *C. chinense* PBC932. All the correlations were marginally negative. The only obvious patterns reflected the low overall levels of lesion size or failure to infect *C. chinense* without wounding.

3.2 Pathogenicity of different *Colletotrichum* species

All the *Colletotrichum* species caused anthracnose symptoms in wounded fruit of *C. annuum* 'Bangchang' at both maturity stages but there were significant differences in severity of the symptoms (Table 2). On wound-inoculated fruit, isolates of *C. scovillei* and *C. javanense* caused the highest disease severity, producing large, necrotic lesions with mean lesion sizes from 1.39 to 2.03 cm (Table 2). All the other species were less aggressive with mean lesion sizes less than 1 cm.

In *C. annuum* 'Bangchang' the majority of the *Colletotrichum* species caused smaller lesions on green fruit compared to ripe red fruits, in both inoculation methods (Table 2). *C. karsti*, *C. fructicola*, and *C. siamense* were moderately aggressive on *C. annuum* red and green fruits when they were wounded. On nonwounded fruits all species were less aggressive, with the mean lesion sizes less than 1 cm at both red and green maturity stages. *C. siamense*, *C. tainanense*, *C. makassarense*, and *C. tropicale* produced only very small lesions or no visible symptoms (mean lesion size ≤ 1 mm) 10 days after inoculation, *C. plurivorum* was not pathogenic on red and green fruit even though one red, nonwounded fruit of Bangchang produced a small lesion. On nonwounded fruit *C. javanense* and *C. scovillei* isolates were the most aggressive on red fruit while *C. scovillei*, *C. karsti*, and *C. javanense* were the most aggressive on green fruit.

In *C. chinense* PBC932, all the species produced smaller lesions than in *C. annuum* 'Bangchang' at both the red and green maturity stages and both inoculation methods. All the *Colletotrichum* species caused moderate anthracnose symptoms on wounded fruit except for *C. plurivorum* (both red and green), *C. tropicale* (green), and *C. fructicola* (green), which were not pathogenic. On wounded fruit, isolates of *C. scovillei*, *C. endophyticum*, and *C. siamense* showed the most severe disease symptoms on red fruit, while *C. scovillei* and *C. javanense* on green fruit produced medium sized, necrotic lesions. All the other species were less pathogenic or did not produce visible symptoms, with the mean lesion sizes less than 0.4 cm 10 DAI.

On nonwounded fruit of *C. chinense* PBC932, there was no significant difference in lesion sizes between any of the species at either maturity stage. All the species were less aggressive (mean lesion size < 1 cm) and *C. fructicola*, *C. makassarense*, *C. tropicale*, and *C. plurivorum* were nonpathogenic at both fruit maturity stages. On green fruit most of the *Colletotrichum* species were not pathogenic except for a few isolates of *C. scovillei*, *C. javanense*, and *C. karsti* which caused small lesions less than 1 cm in some replicates.

3.3 Pathogenicity of *C. scovillei* isolates

All the isolates of *C. scovillei* were highly aggressive on the susceptible *C. annuum* 'Bangchang', regardless of inoculation method and maturity stage, except for isolates UOM 1111 and UOM 1112 from Thailand that did not infect or produced very small lesions on nonwounded red fruit (Table 3). In *C. chinense* PBC932, all the isolates infected wounded fruit, with isolates being generally more aggressive on red than green fruit.

Two isolates from Indonesia (CPC 28615 and CPC 28617) and two from Thailand (UOM 1102 and UOM 1105) were pathogenic on green, nonwounded fruit of *C. chinense* but were not pathogenic on red, nonwounded fruit, except for isolate UOM 1105, which was moderately pathogenic on both red and green nonwounded fruit. Isolates from Malaysia (UOM 1150, UOM 1151, and UOM 1141) and Thailand isolates UOM 1101 and UOM 1105 were pathogenic on nonwounded *C. chinense* red fruit. Considering the differences in pathogenicity of each isolate between Bangchang and PBC932, there were three pathotypes identified on nonwounded green fruit and four in nonwounded red fruit, according to the qualitative differences in lesion development in a specific host condition. There were no pathotypes identified on both green and red wound inoculated fruit.

3.4 Pathogenicity of *C. siamense* isolates

Tests for the 10 isolates of *C. siamense* from Thailand showed that all were pathogenic at red maturity stage of wounded fruits of both *C. annuum* 'Bangchang' and *C. chinense* PBC932 (Table 4). Isolates infecting *C. annuum* were generally more aggressive than on *C. chinense* except for isolate F41C from Thailand which had larger lesion sizes on *C. chinense*. In nonwounded, green fruit of *C. annuum*, three isolates were clearly showing pathotype differences. In *C. annuum* wound-inoculated green fruit three isolates could not infect, whereas seven were pathogenic. Isolates F51A and F54A were highly pathogenic on both *C. annuum* and *C. chinense* fruits at both maturity stages when the fruit was wounded; however, they could not infect any fruit without wounding the fruit periderm.

In *C. chinense* PBC932, no isolate caused symptoms in nonwounded green fruit and only three isolates caused symptoms in nonwounded red fruit, indicating pathotype differences. However, even in wounded fruit they all produced smaller lesions than in *C. annuum*, and three isolates produced no symptoms. There were two pathotypes identified on green nonwounded, four on green wounded, and three on red nonwounded fruits (Table 4). No pathotypes were identified on red wounded fruit.

4 Discussion

Fruit maturity, host line, and inoculation method all interact to affect infection and rate of lesion development, so isolate pathogenicity determined by a detached capsicum fruit bioassay depends on the particular combination of these factors and results obtained in one bioassay may not be useful predictors of pathogenicity under another combination of conditions.

C. scovillei isolates showed large variation in aggressiveness on *C. annuum* fruit and were more severe than on *C. chinense*. The positive correlation in pathogenicity of isolates between mature green and red fruit of *C. annuum* 'Bangchang' for both inoculation methods indicated that for this species, relative pathogenicity estimated in a bioassay would not be affected by fruit maturity or inoculation technique. In contrast, in the bioassay on *C. chinense* PBC932 fruit, which was generally more resistant to *Colletotrichum* spp. (Mongkolporn et al., 2010; Mongkolporn & Taylor, 2011), pathogenicity was dependent on the inoculation method and maturity stage of the fruit. The poor correlation between red and green fruit for both inoculation methods may be explained by the low overall level of infection and variation in lesion size in the nonwounded fruit.

Correlations for pathogenicity between the two *Capsicum* lines were effectively zero in all cases, indicating that aggressiveness on *C. annuum* 'Bangchang' cannot be predicted from aggressiveness on *C. chinense* PBC932 and vice versa, regardless of fruit maturity or wounding. Pathogen isolates which were highly aggressive in one host line were not necessarily the most aggressive in the other host line, in other words there was host specialization. Host line, physiological fruit maturation stage, and inoculation method determined the environment in which the pathogen developed. Different *C. scovillei* isolates were specialized in different host environments.

Inclusion of nonwounding inoculation as a testing parameter contrasts to most previous pathogenicity assays of chili, where bioassays were conducted on wound-inoculated fruit (Mahasuk et al., 2013; Mongkolporn et al., 2010). Most of the isolates that were pathogenic on wound-inoculated fruit were not pathogenic or weakly aggressive without wounding, especially on *C. chinense* PBC2. These findings are in accordance with several studies that determined the pathogenicity of *Colletotrichum* spp., *Alternaria* spp., and *Fusarium* spp. on different hosts and found that disease development was faster on wound-inoculated than nonwound-inoculated fruit (Chen et al., 2017; Kim et al., 2007; Nemsa et al., 2012; Rotondo et al., 2012; Sever et al., 2012).

The Asian *Colletotrichum* species associated with anthracnose in chili fruit varied in pathogenicity and aggressiveness in fruit bioassays on the susceptible *C. annuum* and resistant *C. chinense* lines. *C. scovillei* and the newly identified species *C. javanense* (De Silva et al., 2019) were highly aggressive, being able to infect nonwounded fruit at both mature green and red maturity stages. These two species produced symptoms on the resistant genotype *C. chinense* PBC932 even when nonwounded, emphasizing that they were highly pathogenic and were able to infect through the intact cuticle. These species are likely to be a challenge in breeding for resistance, especially in new *Capsicum* lines containing the *C.*

chinense resistance genetic background. *C. scovillei* is considered the second most important pathogen of *Capsicum* (after *C. truncatum*) and has been isolated frequently in many countries around the world (De Silva et al., 2019; Mongkolporn & Taylor, 2018).

All species were more aggressive on the susceptible cultivar *C. annuum* 'Bangchang' than the relatively resistant genotype *C. chinense* PBC932. In addition, on *C. chinense* PBC932 all the species were moderately or weakly aggressive when the fruit was wounded; however, they were very weakly aggressive or nonpathogenic on nonwounded fruit at both maturity stages. This demonstrated that, apart from a few highly aggressive species (*C. scovillei* or *C. javanense*), most of the other *Colletotrichum* species are unlikely to be important pathogens of *C. chinense* PBC932. These results also agree with the previous bioassays conducted with different *Colletotrichum* species (De Silva et al., 2019; Diao et al., 2017; Liu et al., 2016; Mongkolporn et al., 2010).

C. plurivorum was only weakly aggressive or nonpathogenic in most of the assay conditions except for red, wound-inoculated *C. annuum*. In contrast, Diao et al. (2017) reported that *C. plurivorum* produced moderate to severe symptoms at both maturity stages of all the *Capsicum* genotypes (seven genotypes of *C. annuum*, *C. frutescens*, and *C. chinense*) with wound inoculation. The contradiction between the two studies may have been because different *Capsicum* genotypes/lines were used in the bioassays or the Chinese isolates may have been much more aggressive than the Thai isolates. However, both studies concurred in that the species *C. scovillei*, *C. karsti*, and *C. fructicola* were equally aggressive on *C. annuum*.

C. endophyticum (UOM 1137/ F5 2D) was moderately aggressive on both host genotypes with wound-inoculation and had previously been classified in the most virulent *C. siamense* pathotype group (PCg1-R) by Mongkolporn et al. (2010). In contrast, Diao et al. (2017) found *C. endophyticum* did not infect any of the *Capsicum* species or cultivars they tested. Manamgoda et al. (2013) identified *C. endophyticum* as a weak pathogen of *Pennisetum purpureum* and described it as an endophyte. These findings suggest that the pathogenicity of *C. endophyticum* needs to be confirmed with multiple isolates in further bioassays.

Pathotype differences among the *C. scovillei* isolates were found when tested on nonwound-inoculated fruit. Lack of pathotypic variation in wounded host environments in both maturity stages indicates the importance of nonwound inoculation as a testing condition for identification of pathotype variation within a pathogen population. The pathotypes were not restricted to just one country and occurred in isolates from Indonesia, Malaysia, and Thailand. There were two isolates from Ratchaburi in Thailand that were unable to infect red nonwounded fruit of both genotypes, illustrating the high variability in pathogenicity within *C. scovillei*.

Of the 15 isolates of *C. scovillei*, six isolates (UOM 1101, UOM 1102, UOM 1103, UOM 1104, UOM 1105, and UOM 1107) were previously assessed by Mongkolporn et al. (2010) for pathotype differences. Although these six isolates were highly aggressive on wounded red fruit of both *Capsicum* lines, isolates UOM 1101, UOM 1102, and UOM 1103 were only moderately aggressive on *C. chinense* green fruit. In contrast, in a wounded fruit bioassay, Mongkolporn et al. (2010) found these three isolates to be highly aggressive (severity scores 9) on *C. chinense* but moderately aggressive (severity scores 5–7) on *C. annuum* at both maturity stages.

In addition, all the *C. scovillei* isolates were moderately pathogenic on nonwounded *C. annuum* regardless of the maturity stage and most were not pathogenic on nonwounded *C. chinense* fruit, confirming the relative resistance of *C. chinense* PBC932 to *C. scovillei* and the importance of the cuticle in resistance. Even though both studies used the same isolates and the same *Capsicum* lines, the differences in aggressiveness between the two studies could have been due to small differences in the inoculation technique or the incubation conditions (humidity, temperature), or the scoring system used to assess host infection. Pathogenicity and disease severity of *Colletotrichum* isolates were measured based on quantitative measurements on the lesion size that incorporated all replicated fruit reactions including where there was no lesion (zero). However, Mongkolporn et al. (2010) used an ordinal disease severity rating score (0, 3, 5, 7, and 9) with each rating, depending on a range of lesion sizes, which was a more subjective method of measuring the aggressiveness of the pathogens.

Pathotype differences among the *C. siamense* isolates were identified in the different host environments of the bioassay except in wounded red fruit. This may have been due to differences in resistance gene expression between red and green fruit as reported by Mahasuk et al. (2013) in *C. baccatum*, with red fruit being more susceptible to *C. siamense* isolates. In contrast to *C. scovillei*, *C. siamense* showed pathotype differences in wounded green fruit that illustrated the high variability in interaction between resistance gene expression and virulence among *C. siamense* isolates. Taxonomy and phylogenetic analyses of *C. siamense* isolates from different host species and geographical locations have shown high intraspecific variation within the species, suggesting that *C. siamense* may in fact be composed of several subspecies (De Silva et al., 2017a, 2019; Weir et al., 2012) and hence have different pathogenicity factors controlling pathogenicity.

The six *C. siamense* isolates (UOM 1121, UOM 1125, UOM 1126, UOM 1127, UOM 1128, UOM 1129) previously assessed by Mongkolporn et al. (2010) were all able to infect wounded red fruit of both lines but showed pathotype differences in wounded green fruit. In contrast, Mongkolporn et al. (2010) found none of the isolates were pathogenic on *C. chinense* fruit that were wounded at both maturity stages, and at the green stage of *C. annuum*. Differences in pathogenicity of *C. siamense* isolates between the two studies may have been due to the same reasons explained for *C. scovillei*, as the bioassays were carried out in different laboratories and with different scoring systems.

In summary, the inoculation method was the most important factor in conducting a detached fruit pathogenicity assay for anthracnose of chili. Wounding greatly enhanced the ability of *Colletotrichum* to cause disease and allowed several weakly pathogenic species (opportunistic pathogens) to infect and cause observable disease. This outcome emphasizes the importance of conducting bioassays with both nonwound and wound inoculation and demonstrates the role of the cuticle as a barrier to pathogen infection of the fruit. Nonwounded inoculation also identifies the variations in genetic structure (pathotype) within pathogen populations, which is important for development of effective control measures for targeted species. Most importantly, the results demonstrate the value of minimizing fruit damage in the field during growing, and at postharvest stages. Oh et al. (1999) also showed the importance of cuticular wax layers of green and red chili fruit to infection by *C. gloeosporioides*, where a negative correlation was found between cuticle thickness and disease incidence.

Anthracnose is a major disease of chili and it is necessary to identify effective strategies for disease control. The identification of variability in pathogenicity and aggressiveness between different *Colletotrichum* species and pathotypes within *C. scovillei* and *C. siamense* is a significant finding that should better inform chili breeding for anthracnose resistance. In addition, this will improve understanding of the specific interactions between chili genotypes, *Colletotrichum* species/isolates, chili fruit maturity stages, and inoculation technique. Further studies in identification of resistance gene loci to a broad range of *Colletotrichum* species and pathotypes will be important in developing successful resistant cultivars.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figure legends

Figure 1 Correlation plots categorized by two of the factors and their consistency between the two levels of the remaining factor. Each dot point represents least squares means of lesion size and their 95% confidence intervals estimated at each testing environment. Comparison of isolate mean lesion length between (a) wounding and nonwounding inoculations for each fruit ripeness and host line combination; (b) mature green and red fruit for each inoculation technique and host line combination; and (c) *Capsicum chinense* PBC932 and *Capsicum annuum* ‘Bangchang’ for each inoculation technique and fruit ripeness combination.

Table 1 Colletotrichum species and isolates used for the pathogenicity assay and their geographic locations

Colletotrichum species	Species complex	Isolate	Collection location
C. endophyticum	gloeosporioides	UOM 1137 (F5-2D)[a]	Thailand
C. fructicola	gloeosporioides	CPC 28644, UOM 1138	Thailand, Taiwan
C. javanense	acutatum	UOM 1115	Indonesia
C. karstii	boninense	CPC 28553, CPC 28554, CPC 28602	Indonesia
C. makassarense	gloeosporioides	CPC 28555, CPC 28556, CPC 28612	Indonesia
C. plurivorum	orchidearum	UOM 1004, UOM 1005, UOM 1006, CPC 28638, CPC 28639	Thailand
C. scovillei	acutatum	CPC 28615	Maros, Indonesia
		CPC 28617	
		CPC 30224	Gowa, Indonesia
		UOM 1150 (4-46-3D)	Pahang, Malaysia
		UOM 1141 (A15)	
		UOM 1151 (E15)	Johor, Malaysia
		UOM 1140 (F59)	Kelantan, Malaysia
		UOM 1101 (313)[a]	Chiang Mai, Thailand
		UOM 1102 (322)[a]	
		UOM 1103 (311)[a]	
		UOM 1104 (314)[a]	
		UOM 1105 (MJ3)[a]	
		UOM 1107(MJ7)[a]	
		UOM 1111 (GA1)	Kasetsart University site,
		UOM 1112 (GA2)	Nakhon Pathom, Thailand
C. siamense	gloeosporioides	UOM 1121 (F4-1B)[a]	Kanchana Buri, Thailand
		UOM 1126 (F4-1C)[a]	

		UOM 1127 (F51A)[a]	
		UOM 1129 (F54A)[a]	
		UOM 1128 (F7-1B)[a]	Nakhon Pathom, Thailand
		UOM 1125 (F7-3B)[a]	
		UOM 1132 (RC-1a)	Ratchaburi, Thailand
		UOM 1133 (RC-1b)	
		UOM 1134 (RC-1C)	
		UOM 1135 (RC-2b)	
C. tainanense	gloeosporioides	UOM 1119, UOM 1120	Taiwan
C. tropicale	gloeosporioides	CPC 28607, UOM 1002, UOM 1003	Indonesia

[a]Isolates used in the pathogenicity assays of Mongkolporn et al. (2010).

Table 2 Least squares mean diameter (cm) of lesions caused by different Colletotrichum species in different test conditions (two maturity stages, two Capsicum lines, with two inoculation methods)

	Capsicum annuum 'Bangchang'				**Capsicum chinense PBC932**				
	NW		**W**		**NW**		**W**		
Colletotrichum species	**Red**	**Green**	**Red**	**Green**	**Red**	**Green**	**Red**	**Green**	**Average**
C. javanense	0.94 a	0.33 ab	1.56 ab	1.39 ab	0.10 a	0.10 a	0.39 bcd	0.26 ab	0.64
C. scovillei	0.91 ab	0.70 a	2.03 a	1.67 a	0.10 a	0.05 a	1.34 a	0.69 a	0.94
C. fructicola	0.36 bc	0.13 b	0.78 bcd	0.24 cd	0.00 a	0.00 a	0.23 bcd	0.00 b	0.22
C. endophyticum	0.28 bc	0.06 b	0.44 cd	0.28 cd	0.02 a	0.00 a	0.74 abc	0.18 ab	0.25
C. karsti	0.20 c	0.39 ab	0.94 bcd	0.64 bc	0.10 a	0.02 a	0.36 bcd	0.14 b	0.35
C. tainanense	0.15 c	0.11 b	0.69 bcd	0.56 bcd	0.10 a	0.00 a	0.34 bcd	0.16 b	0.26
C. siamense	0.13 c	0.05 b	0.97 bc	0.27 cd	0.04 a	0.00 a	0.55 b	0.12 b	0.27
C. makassarense	0.06 c	0.11 b	0.64 cd	0.54 cd	0.00 a	0.00 a	0.18 cd	0.09 b	0.20
C. tropicale	0.02 c	0.04 b	0.41 d	0.14 cd	0.00 a	0.00 a	0.11 cd	0.00 b	0.09
C. plurivorum	0.01 c	0.00 b	0.53 d	0.08 d	0.00 a	0.00 a	0.00 d	0.00 b	0.08

Note. NW, nonwounded inoculation, W, wounded inoculation.

Significant differences at α = .05 are indicated by different letters according to Fisher pairwise comparison between species least squares means within each column.

Table 3 Least squares mean diameter (cm) of lesions caused by isolates of Colletotrichum scovillei in particular host by inoculation method combinations

		Green/NW		Green/W		Red/NW		Red/W	
Country	**Isolate**	**Ca Bn**	**Ch 93**	**Ca Bn**	**Ch 932**	**Ca Bn**	**Ch 932**	**Ca Bn**	**Ch 932**
Indonesia	CPC 28615	0.89	0.17	2.28	0.25	0.83	0.00	2.00	1.22
	CPC 28617	0.94	0.08	1.94	0.83	0.72	0.00	1.20	1.33
	CPC 30224	0.94	0.00	1.56	0.42	0.30	0.00	1.60	0.67
Malaysia	UOM 1150, 4-46-3D	0.83	0.00	1.94	0.58	0.30	0.17	1.70	1.11
	UOM 1151, E15	1.46	0.00	2.04	1.33	0.56	0.33	2.20	0.52
	UOM 1140, F59	1.33	0.00	2.08	1.00	0.44	0.00	2.10	1.28
	UOM 1141, A15	0.44	0.00	0.94	1.75	0.44	0.39	1.70	1.78
Thailand	UOM 1101, 313	0.75	0.00	1.50	0.17	0.11	0.28	1.70	1.58
	UOM 1102, 322	0.33	0.17	1.72	0.33	0.17	0.00	1.70	1.44
	UOM 1103, 311	0.39	0.00	1.44	0.33	0.06[a]	0.00	1.90	1.67
	UOM 1104, 314	0.22	0.00	1.28	0.83	0.72	0.00	2.10	1.63
	UOM 1105, MJ3	0.44	0.33	1.44	1.08	0.06[a]	0.11	1.60	1.67
	UOM 1107, MJ7	1.17	0.00	2.33	0.75	0.76	0.00	1.60	1.56
	UOM 1111, GA1	0.06[a]	0.00	1.50	0.25	0.00	0.00	1.40	1.14
	UOM 1112, GA2	0.11	0.00	0.83	0.50	0.00	0.00	0.90	1.44
Pathotype		3		0		4		0	

Note. Ca Bn, Capsicum annuum 'Bangchang'; Ch 932, Capsicum chinense PBC932; NW, nonwounded inoculation; W, wounded inoculation.

[a]Symptoms caused by 1 out of 9 replicates, considered as mean lesion size of 0 or no symptoms.

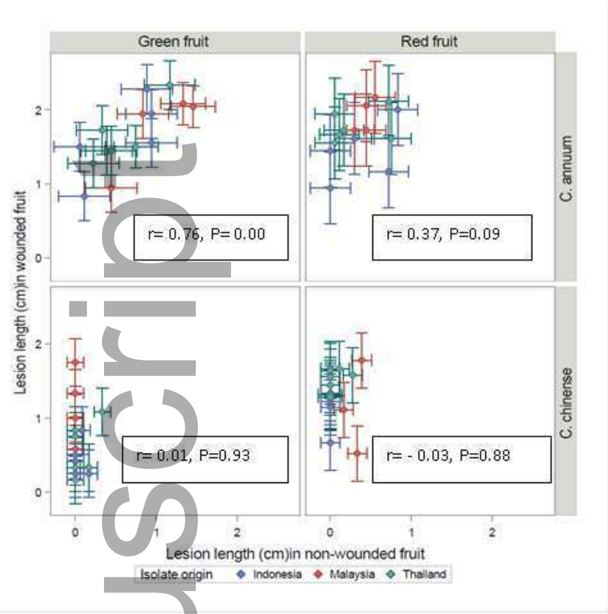
Table 4 Least squares mean lesion diameter (cm) of lesions caused by isolates of Colletotrichum siamense in particular host by inoculation method combinations

Isolate	Green/NW		Green/W		Red/NW		Red/W	
	Ca Bn	**Ch 932**	**Ca Bn**	**Ch 932**	**Ca Bn**	**Ch 932**	**Ca Bn**	**Ch 932**
UOM 1121, F41B	0.00	0.00	0.00	0.15	0.11	0.00	1.11	0.22
UOM 1126, F41C	0.00	0.00	0.50	0.00	0.06[a]	0.00	0.29	0.50
UOM 1127, F51A	0.00	0.00	0.11	0.13	0.00	0.00	1.33	0.89
UOM 1129, F54A	0.00	0.00	0.11	0.18	0.00	0.00	1.78	1.33
UOM 1128, F71B	0.00	0.00	0.00	0.00	0.17	0.00	1.11	0.89
UOM 1125, F73B	0.00	0.00	0.00	0.35	0.14	0.00	0.67	0.21
UOM 1132, RC1a	0.00	0.00	0.17	0.00	0.11	0.00	1.06	0.09
UOM 1133, RC1b	0.09	0.00	0.26	0.15	0.17	0.16	0.89	0.33
UOM 1134, RC1C	0.14	0.00	0.67	0.08	0.18	0.14	0.78	0.47
UOM 1135, RC2b	0.28	0.00	0.89	0.20	0.39	0.06	1.67	0.56
Pathotype	2		4		3		0	

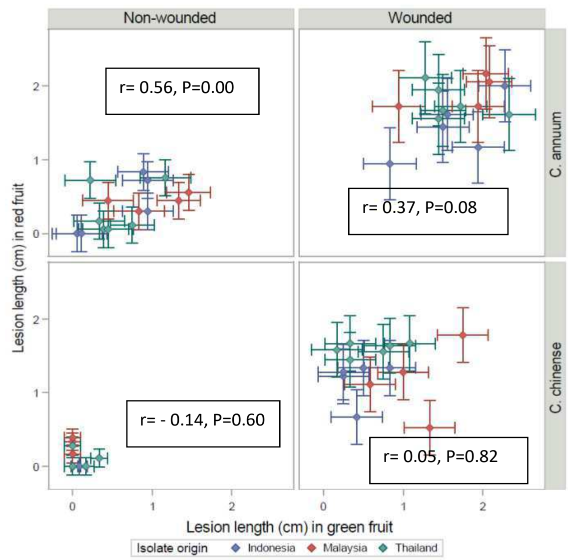
Note. Ca Bn, Capsicum annuum 'Bangchang'; Ch 932, Capsicum chinense PBC932; NW, nonwounded inoculation; W, wounded inoculation.

[a]Symptoms caused by 1 out of 9 replicates, considered as mean lesion size of 0 or no symptoms.

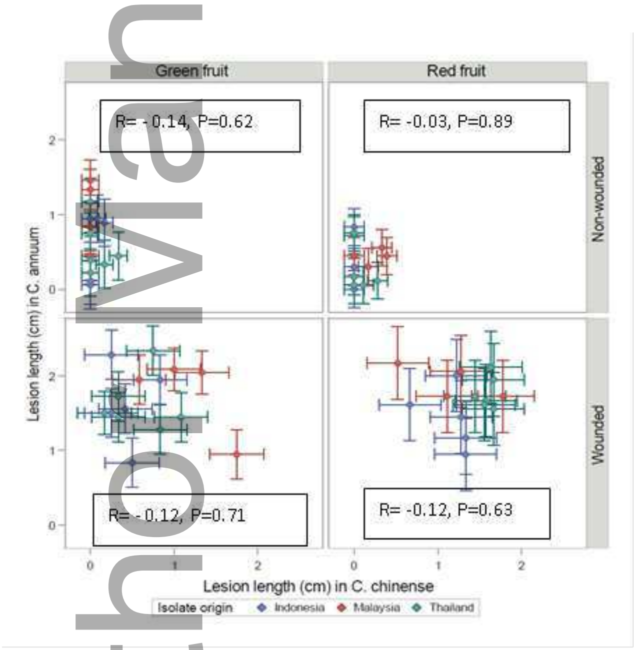
a)


Green fruit
Red fruit
C. annuum
C. chinense
r= 0.76, P= 0.00
r= 0.37, P=0.09
r= 0.01, P=0.93
r= - 0.03, P=0.88
Lesion length (cm)in wounded fruit
Lesion length (cm)in non-wounded fruit
Isolate origin
Indonesia
Malaysia
Thailand

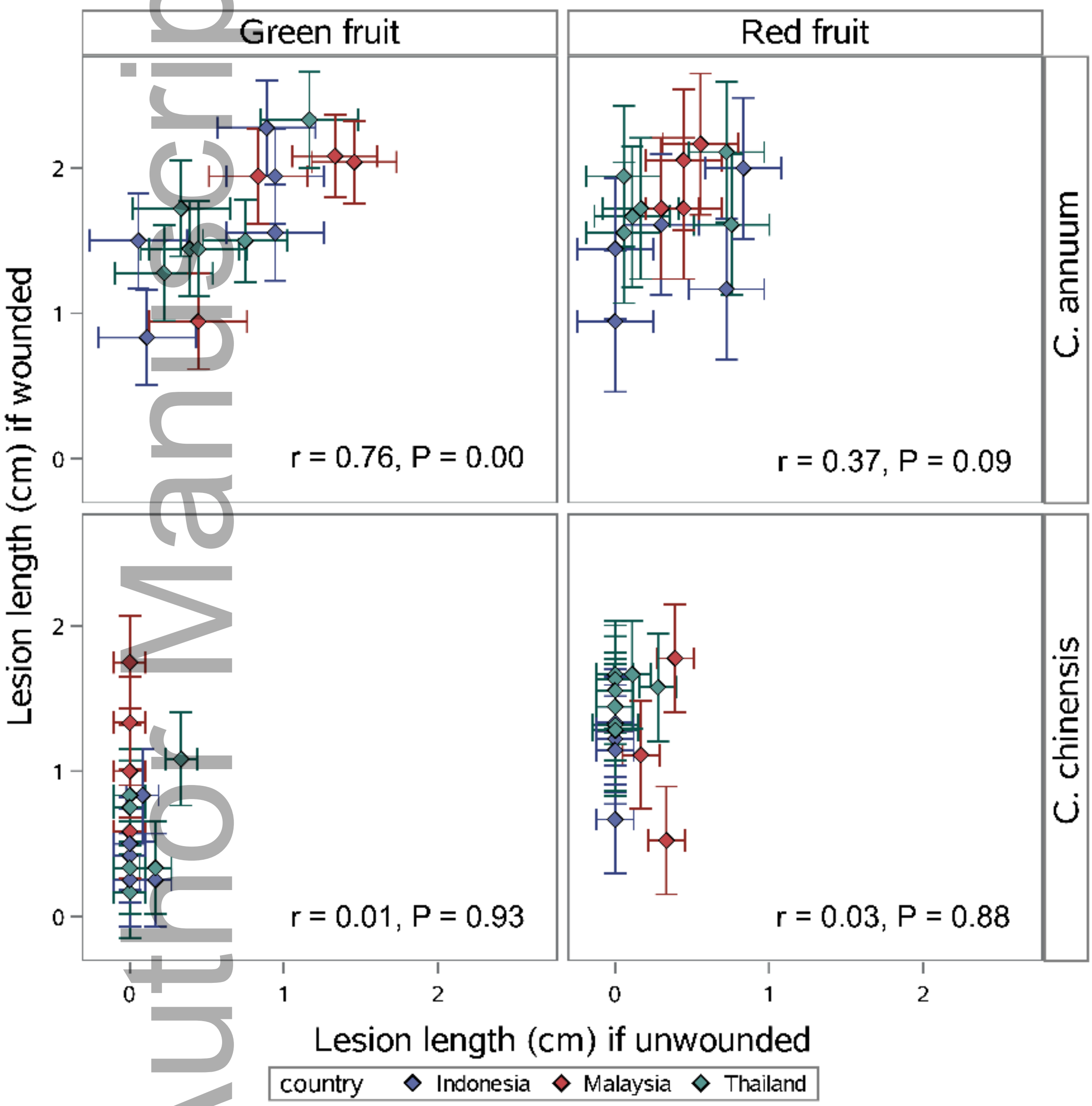
b)


Non-wounded
Wounded
C. annuum
C. chinense
r= 0.56, P=0.00
r= 0.37, P=0.08
r= - 0.14, P=0.60
r= 0.05, P=0.82
Lesion length (cm) in red fruit
Lesion length (cm) in green fruit
Isolate origin
Indonesia
Malaysia
Thailand

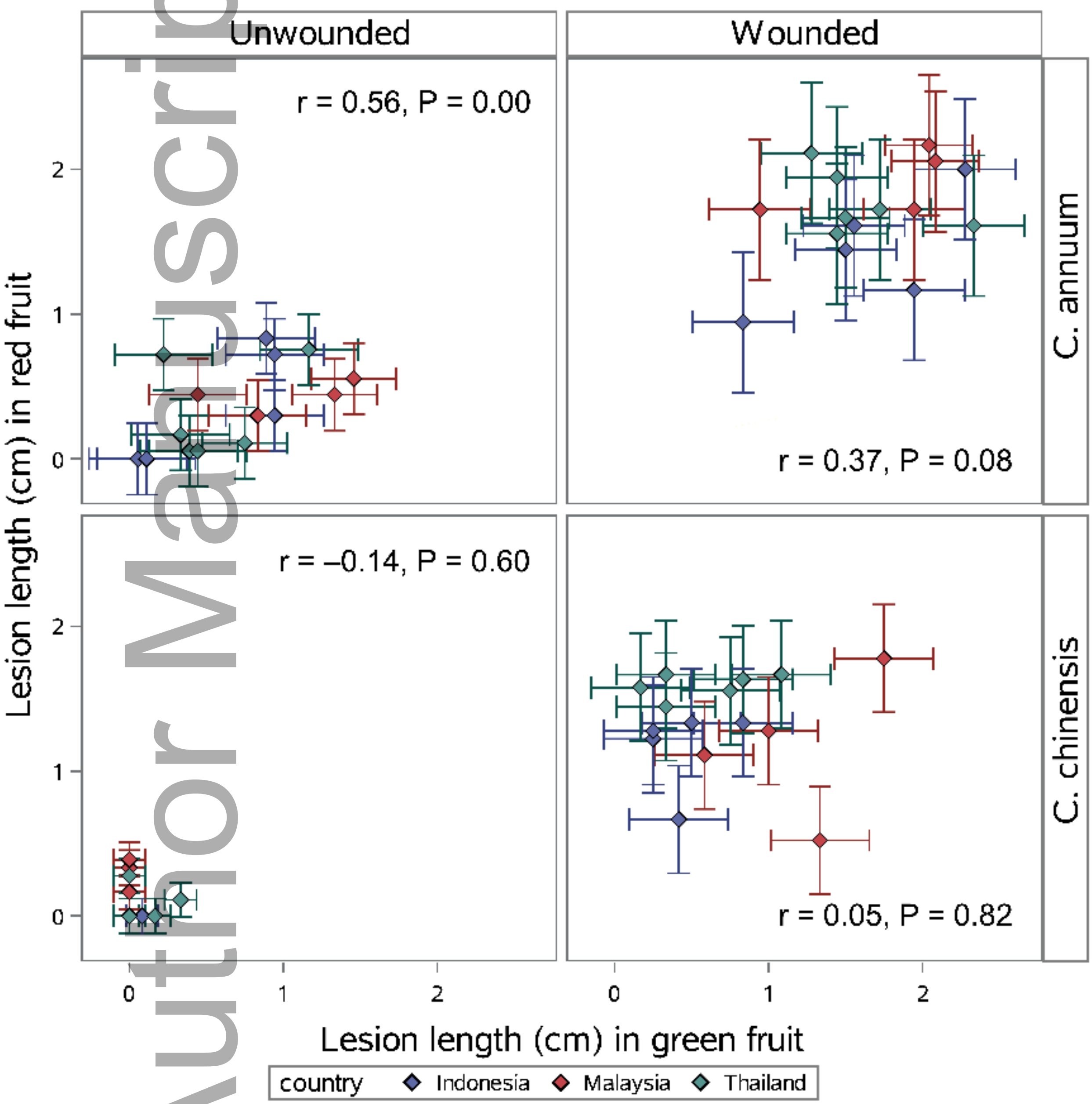
c)


Green fruit
Red fruit
Non-wounded
Wounded
R= - 0.14, P=0.62
R= -0.03, P=0.89
R= - 0.12, P=0.71
R= -0.12, P=0.63
Lesion length (cm) in C. annuum
Lesion length (cm) in C. chinense
Isolate origin
Indonesia
Malaysia
Thailand

(a)

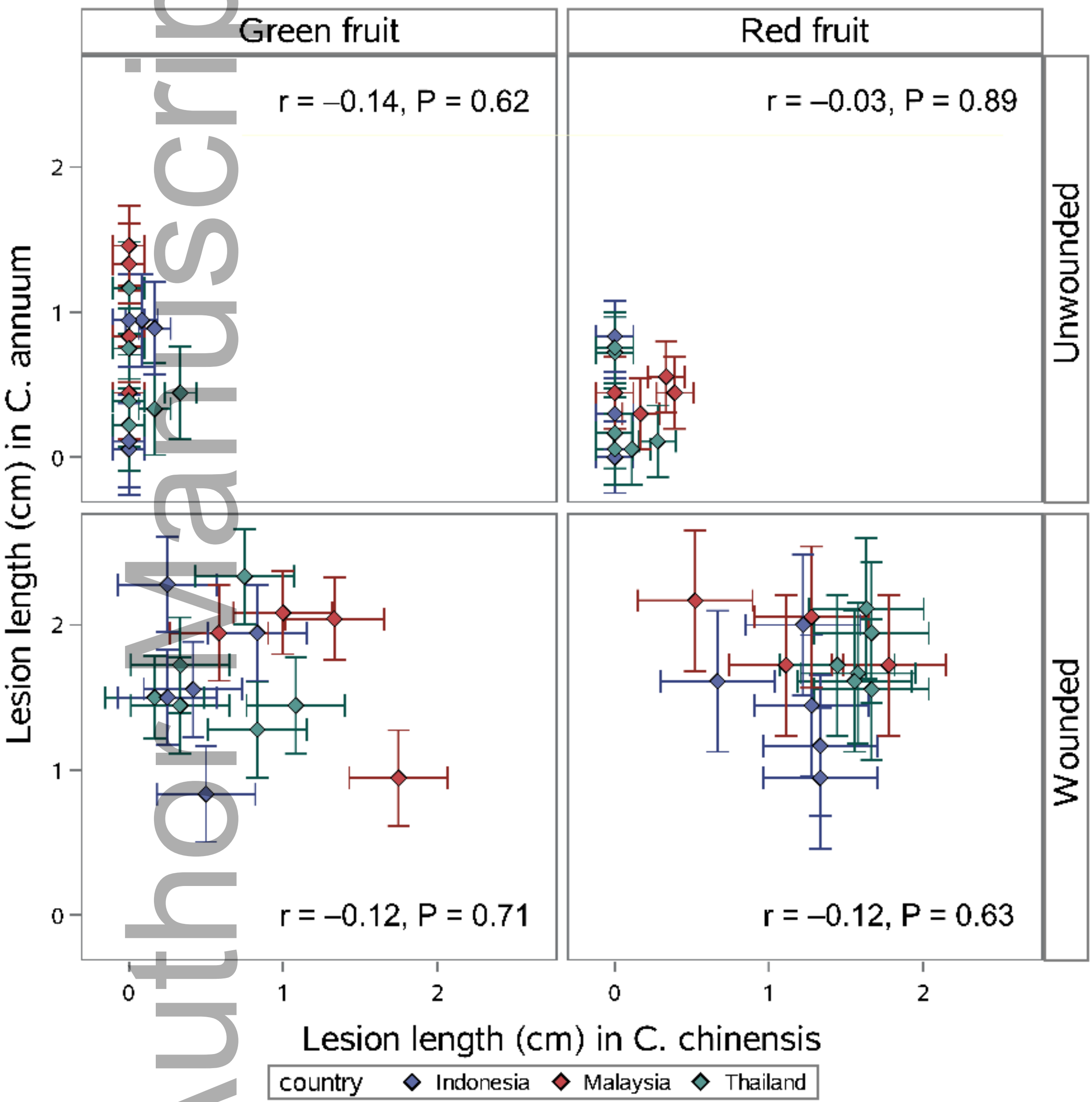

Green fruit
Red fruit
C. annuum
C. chinensis
Lesion length (cm) if wounded
Lesion length (cm) if unwounded
r = 0.76, P = 0.00
r = 0.37, P = 0.09
r = 0.01, P = 0.93
r = 0.03, P = 0.88
0
1
2
country
Indonesia
Malaysia
Thailand

(b)



Unwounded
Wounded
r = 0.56, P = 0.00
r = 0.37, P = 0.08
r = –0.14, P = 0.60
r = 0.05, P = 0.82
C. annuum
C. chinensis
Lesion length (cm) in red fruit
Lesion length (cm) in green fruit
0
1
2
country
Indonesia
Malaysia
Thailand

(c)



Green fruit
Red fruit
Unwounded
Wounded
r = –0.14, P = 0.62
r = –0.03, P = 0.89
r = –0.12, P = 0.71
r = –0.12, P = 0.63
Lesion length (cm) in C. annuum
Lesion length (cm) in C. chinensis
0
1
2
country
Indonesia
Malaysia
Thailand