CHAPTER 4
RESULTS

4.1 Isolation of white and pigmented *C. parasitica* from cankers on the grafted trees

In 1998 and 1999 a total of 49 superficial cankers, located outside of the H-inoculated zone, were sampled on the TH, RM and TG grafts. For each graft, cankers were sampled at distances ranging from less than 100 cm from the H-inoculated zone to 564 cm (TH), 619 cm (RM), and 494 cm (TG) outside of the H-inoculated zone. Of the 49 cankers sampled, 43 were main stem cankers and six were branch cankers. The position of cankers and number of white and pigmented isolates recovered are recorded in Table 4.1.

All cankers sampled yielded *C. parasitica* isolates, but white isolates were recovered from 85.7% of the cankers. A total of 202 *C. parasitica* isolates were recovered from the cankers, and the number of white isolates recovered from each canker ranged from zero to four. Of these 202 isolates, 95 (47%) were white and 107 (53%) were pigmented isolates. The TH graft yielded the most white isolates 38/76 (50%) followed by RM (34/70 or 49%) and TG (23/56 or 41%).

Table 4.1. Number of white and pigmented isolates of *Cryphonectria parasitica* collected from the 49 cankers sampled at various distances from the hypovirulent strain inoculated zone on grafted American chestnuts at Lesesne State Forest

	Distance (cm)[a]	Graft[b]	W/P[c]	VC groups[d]	Distance (cm)	Graft	W/P	VC groups
Cankers								
	47	THL	2/2	XVI,single	325	RML	3/1	VI
	67	TG	1/5	single	327	THR	1/2	II
	87	THR	2/1	IX,single	330	THL	2/0	I
	102	RML	5/1	XX,XXIII	342	RML	1/0	single
				XXV,single	356	TG*	3/3	XIII
	152	THL	2/4	XVIII	362	RMR	0/2	
	157	THR	1/3	XVII	368	TG	3/0	XII
	167	RMR	¼	XX	369	THL	2/2	I
	187	RML	4/2	XXII,XXIV	385	TG	4/2	XI
				single	389	THL	4/1	VIII,XI
	199	TG	3/2	III,XIX	392	RMR	3/0	X,single
	207	THL*	1/3	single	398	THR	1/3	II
	219	THR	3/1	XVI	417	RMR	0/2	
	237	RMR*	4/2	I,XXI, single	427	RML	3/2	XVII,
	242	THL	4/1	VII,XVII				single
				XVII	432	THL	2/4	VII
	254	TG	2/1	XIV	437	RML	4/1	V
	271	THR	2/2	II,single	453	THR	2/1	II
	276	THL	1/0	I	455	THR	0/1	
	287	TG*	3/2	XV	474	RMR	1/5	XXIV
	287	TG*	2/4	XVII,single	476	TG*	2/3	XIX
	288	TG	0/5		494	TG	0/6	
	296	THL	3/1	I,single	505	THR	1/3	single
	301	THL	0/1		602	RML	1/1	IV
	311	RMR	2/1	XXI,single	619	RMR	2/4	XXIV
	317	THL	1/0	I	642	RML	0/6	

[a] Distance from the zone inoculated with hypovirulent strains in 1982 – 1983 on each grafted American chestnut.
[b] Graft and stem (left or right) from which canker was sampled. THL, RML = left stem; THR, RMR = right stem.
[c] W/P = number of white isolates/ number of pigmented isolates of *C. parasitica* recovered from each canker.
[d] Vegetative compatibility groups identified for isolates recovered from each canker
* Branch canker

4.2 Vegetative compatibility of white *C. parasitica* isolates

Vegetative compatibility tests were performed for each of the 95 white isolates collected following single-spore isolation of the pigmented form of the clone. In addition, to these white isolates, 15 more white isolates from the grafts were added to the vegetative compatibility tests. These isolates were collected from the TH, RM, and TG grafts, in 1997 and 1998 by Robbins and Griffin (1999). The isolates were paired in all possible combinations. Forty-eight VC groups were identified among the 110 white isolates. The diversity of vegetative compatibility groups was calculated using the ratio of VC groups to isolates tested (S/N) and the Shannon diversity index (Anagnostakis 1986). The S/N ratio was calculated to be 0.43 and the Shannon diversity index was 3.64.

A total of 25 VC groups were identified with two or more isolates in each VC group and 23 with one isolate each (Table 4.2). Of these 25 groups, VC group I was the largest with 10 isolates. This group was present in both the TH and RM grafts, while VC group IV was common to the RM and TG grafts, and XVII was common to all three trees. The TH graft had the lowest ratio of VC groups to isolates tested (S/N = 0.40) and the RM graft had both the largest number of VC groups (22) and the highest S/N ratio (0.58) (Table 4.2).

The 25 major VC groups were also paired against 11 of the most common pigmented VC groups collected by Robbins and Griffin (1999) from 1997-1998 and the four original inoculated hypovirulent strains (EP-43, EP-47, EP-49, EP-51) obtained from ATCC. None of the four ATCC cultures were compatible with any of the 25 major white VC groups; however, five of the 11 pigmented VC groups were compatible with the major VC groups. Robbins and Griffin (1999) collected 28 pigmented VC groups from 1997-1998. Excluding the five common groups, there are a possible 71 VC groups present in

the grafts at Lesesne. These putative VC groups are comprised from 175 white and pigmented isolates, yielding a S/N ratio of 0.41.

Table 4.2. Number of major VC groups (those with two or more isolates) of white *Cryphonectria parasitica* in each American chestnut graft along with corresponding number of isolates in each VC group from that graft. VC groups common to more than one tree are in **bold**. Single isolate groups are presented for each graft along with the total number of VC groups and VC ratio

TH graft			RM graft			TG graft		
VC group[a]	No. of cankers[b]	No. of isolates[c]	VC group	No. of cankers	No. of isolates	VC group	No. of cankers	No. of isolates
I	6	9	**I**	1	1	III	2	3
II	4	5	**IV**	1	1	**IV**	1	1
VII	2	3	V	1	4	XI	1	4
VIII	1	2	VI	1	3	XII	1	3
IX	2	3	X	1	2	XIII	1	3
XVI	3	5	**XVII**	1	2	XIV	1	2
XVII	2	2	XX	2	2	XV	2	4
XVIII	2	4	XXI	2	3	**XVII**	1	1
			XXII	1	2	XIX	3	4
			XXIII	1	2			
			XXIV	3	4			
			XXV	2	2			
[d]Single isolate groups		9			10			4
Total VC groups		17			22			13
Total no. white isolates		43			38			29
[e]VC ratio (S/N)		0.395			0.579			0.448

[a] White VC groups with more than one isolate in cankers on the TH, RM and TG grafted American chestnut trees.
[b] Number of cankers containing recovered isolates identified to VC group.
[c] Number of isolates present in the white VC groups containing more than one isolate in cankers on the three grafted American chestnut trees.
[d] Number of white VC groups containing only one isolate in cankers on the three grafted American chestnuts.
[e] Ratio of white VC groups to total number of white isolates present in the three grafted American chestnuts.

4.3 Cultural characteristics of white *C. parasitica* isolates

The four hypovirulent isolates of *C. parasitica* that were inoculated in the grafts in 1982 and 1983, were obtained from ATCC, grown singly on APDA, and the colonies were compared at 7 days with pictures from Elliston (1985). All but one of the isolates was similar to Elliston's original descriptions from 1985. The one isolate, EP-51, produced a moderately pigmented, slow growing colony with a wavy margin. Elliston's pictures show EP-51 as a fast growing white isolate, with little to no pigment. This colony type was later recovered following isolation of single spores from the ATCC EP-51 isolate, and designated EP-51W. In addition, although the EP-43 isolate was classified as white at 7 days by Elliston (1985), it was highly pigmented at 14 days in the present study and would not be classified as white by the criteria used here.

White single-spore colonies from isolates recovered from cankers on the trees were of predominantly four colony morphology (CM) groups or types at 14 days. Colonies had 1) white centers with white "fast, advancing" margins; 2) white centers with light to dark brown wavy margins; 3) white or lightly pigmented centers with numerous lightly pigmented yellow-orange pycnidia on margins; and 4) lightly yellow-orange, pigmented centers with white "fast, white advancing " margins (Fig 4.1, Table 4.3). Two of the inoculated strains, EP-47 and EP-49, were placed in CM groups 2 and 3, respectively. Single-spore colonies from EP-49 were of both CM 1 (4 colonies) and CM 3 (8 colonies) types. The white single-spore colonies obtained from EP-51 (EP-51W) were classified in CM group 1, whereas the EP-51 isolate obtained from ATCC was loosely classified to CM group 2. Cultural morphology group 2 had characteristics closest to those of EP-51, however EP-51 was slower growing, darker pigmented and had a slightly different margin shape than any of the typical isolates classified to CM group 2. EP-43 was not classified

as white after 14 days because of highly pigmented colonies and therefore did not group with any CM type (Fig 4.2). Furthermore, none of the white isolates recovered from the trees resembled EP-43. Nearly all of the 110 white isolates were classified into the four CM groups. Criteria for colony characteristics used by other researchers are given in Table 4.3. Occasionally, the single spore colonies of the recovered isolates were composed of more than one colony morphology group, or had some colonies that were not typical of any CM group. When this occurred (in about 30% of the white isolates recovered), the isolate was classified to the CM group that was most common to its single spore colonies. Cultural morphology group 3 contained the most recovered isolates (37), followed by CM group 1 (33), CM group 2 (23) and CM group 4 (15). The total number of isolates and VC groups present in each CM group are presented in Tables 4.4 – 4.7.

Table 4.3. Colony morphology criteria used for classification of white and pigmented *Cryphonectria parasitica* strains or isolates, as proposed by Elliston (1985), Coskun et al.(1999), Hogan and Griffin, and Bisseger et al. (1996). Normal strains are those strains considered to be pigmented and virulent while pigmented strains are distinguished only by color

Elliston[a] Mass transfer colonies, one per plate	**Hypovirulent** Rapid or slow growth, abundant or little mycelium, highly or weakly organized leading mycelium; and "white for at least the first 5 days after transfer, then some developed cream, yellow or light orange centers, or light orange concentric rings"			**Normal** "orange color typical of *E. parasitica*", and "well defined concentric rings "	
Coskun et al.[b] Mass transfer colonies, one per plate	**Hypovirulent** "white mycelium and few and large pycnidia"	**Intermediate** "whitish-cream mycelium with pycnidia uniformly distributed over the entire colony"	**Normal** "cream colored mycelium, abundant orange pycnidia scattered within concentric rings and spore tendril production"		
Bisseger et al.[d] Mass transfer colonies, one per plate	**White** "remained white" after growth conditions		**Orange Pigmented** "having clearly discernable orange pigmentation"		
Hogan and Griffin[c] Single-spore colonies, four per plate	**White CM Group 1** "white centers with white 'fast, advancing' margins"	**White CM Group 2** "white centers with wavy light to dark brown margins"	**White CM Group 3** "white or lightly pigmented centers with numerous pigmented pycnidia on margins"	**White CM Group 4** "lightly pigmented centers with white 'fast, advancing ' margins"	**Pigmented** "more than 50% of colony color is pigmented"

[a] Elliston (1985) classification of white (Italian) and normal strains of *C. parasitica* using conditions of inverted cultures " under fluorescent lights and incubated at 20 ± 2 C for 6-7 days with a 16hr photoperiod on potato dextrose agar amended with methionine and biotin.

[b] Coskun et al. (1999)description of hypovirulent, intermediate, and normal strains of *C. parasitica* under conditions of darkness for 7-14 days on potato dextrose agar amended with methionine and biotin.

[c] Hogan and Griffin classification of white colony morphology (CM) groups, and pigmented classification under conditions of daily cool whitefluorescent light and room temperature for 14 days, and grown on acidified potato-dextrose agar (Difco). Inoculated strains EP-51W, EP-51, EP-47, and EP-49 were grouped into CM group 1, CM group 2, CM group 2, and CM groups 3 or 1 respectively.

[d] Bisseger et al. (1996) classification of white and pigmented isolates using growth conditions of "25^{o} C in the dark for 7 days, followed by incubation under daylight on the laboratory bench for 5 days" on potato dextrose agar.

Fig 4.1. Representative 14-day-old colonies of white *Cryphonectria parasitica* single-spore isolates from the four cultural morphology (CM) groups assigned to the isolates recovered from cankers on the American chestnut grafts. Clockwise from top left: CM 1) white centers with white "fast, advancing" margins; CM 2) white centers with light to dark brown wavy margins; CM 3) white or slightly pigmented centers with numerous lightly pigmented yellow-orange pycnidia on margins; and CM 4) lightly yellow-orange pigmented centers with white "fast, advancing " margins

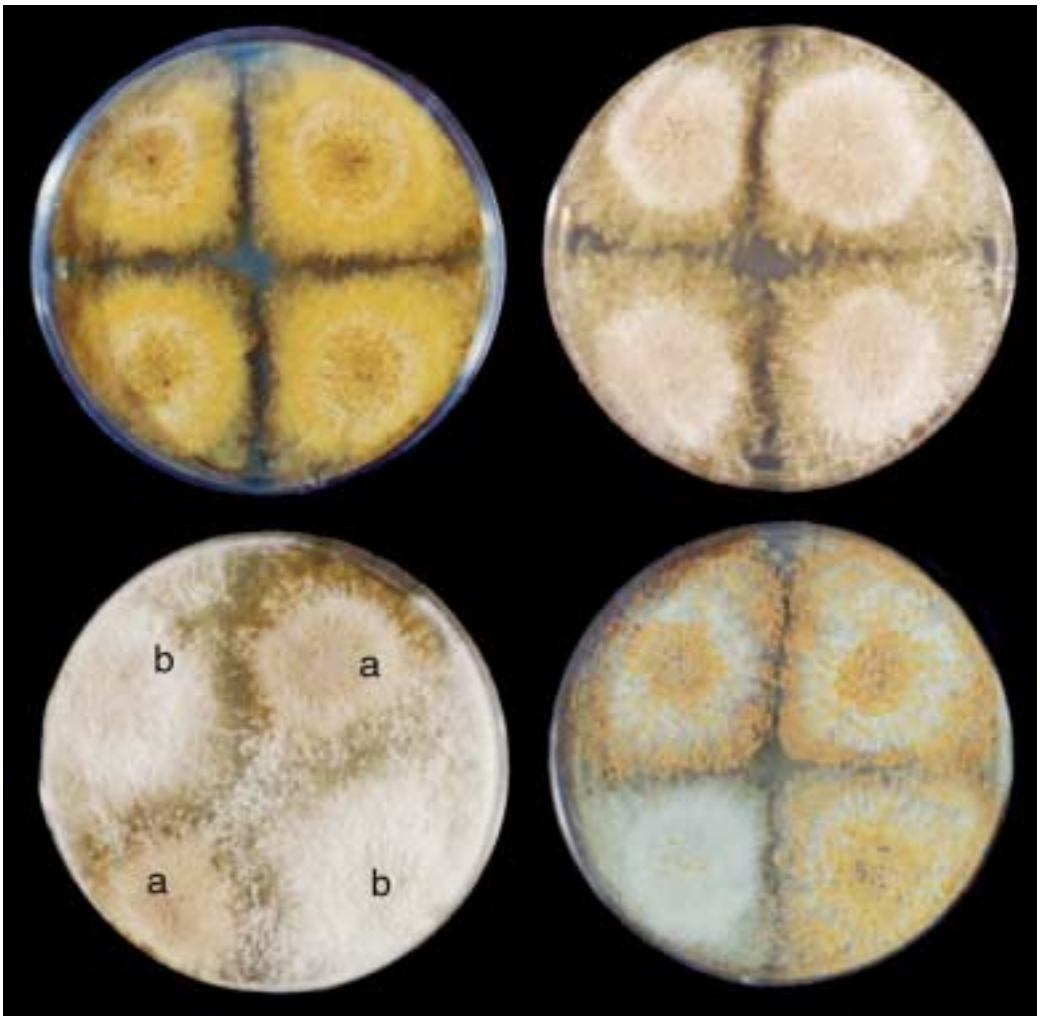


Fig 4.2. Representative 14-day-old single-spore colonies from hypovirulent *Cryphonectria parasitica* strains inoculated on grafted American chestnut trees in 1982 and 1983 (clockwise from top left) EP-43 (ATCC# 38767), EP-47 (ATCC# 38760), EP-49 (ATCC# 38759), EP-51 (ATCC# 38758). Single-spores from EP-49 were assigned to CM groups 1 and 3. Single-spores from EP-47 were assigned to CM group 2. Single-spore colonies from EP- 43 had white margins at 7 days, but were not considered white after 14 days; no recovered isolates resembled this colony morphology type. Most EP-51 single-spore colonies, those that resembled the mass transfer colony (a), were classified in CM group 2. Some single spores however, produced entirely white colonies (labeled in text as EP-51W) after transfer (b), which were placed into CM group 1. This colony type is similar to that described by Elliston for EP-51 (1985)

Table 4.4. White *Cryphonectria parasitica* isolates recovered from cankers on the three grafted American chestnut trees classified with CM group 1 cultural characteristics (white centers and white "fast, advancing" margins) at 14 days on single spore plates

CM group 1

TH graft		**RM graft**		**TG graft**	
Isolate	VC group	Isolate	VC group	Isolate	VC group
THL-747 (TH-747cm)[a]	single[b]	RML-508a	VC VI	TG-659d	VC XIX
THL-615b	VC VIII	RML-508d	VC VI	TG-470a	single[b]
THL-615d	VC VII	RML-370f	VC XXIV	TG-382d	VC III
THL-572b	VC VIII	RML-285c	VCXX	TG-382e	VC III
THL-572c	VC IX	RML-285d	VCXXV	TG-287f	VC XV
THL-572f	VC IX	RML-285e	VC XXIII		
THL-513b	VC I	RMR-802c	VC XXIV		
THL-513d	VC I	RMR-802f	VC XXIV		
THL-425b	VC XVIII	RMR-494b	single[b]		
THL-425c	VC XVII	RMR-420b	VC I		
THL-425d	VC VII	RMR-420c	VC XXI		
THL-425e	VC XVIII	RMR-420e	single[b]		
THR-402b	VC XVI	RMR-420f	VC XXIV		
THR-270c	single[b]	RMR-350b	VC XX		
14 isolates	9 VC groups	14 isolates	9 VC groups	5 isolates	4 VC groups

[a] Isolate collected in 1997- 1998 and renamed with updated nomenclature.
[b] Isolate belongs to a vegetative compatibility group composed of only itself.

Table 4.5. White *Cryphonectria parasitica* isolates recovered from cankers on the three grafted American chestnut trees classified with CM group 2 cultural characteristics (white centers with light to dark brown wavy margins) at 14 days on single spore plates

CM group 2

TH graft		**RM graft**		**TG graft**	
Isolate	VC group	Isolate	VC group	Isolate	VC group
THL-552a	VC I	RML-285a	single[b]	TG-568c	VC XI
THL-552d	VC I	RML-109		TG-551c	VC XII
THL-527b	VC I	(3A)[a]	single[b]	TG-287a	VC XV
THL-500b	VCI	RMR-657f	VC XXIV	TG-157	
THL-479c	single[b]	RMR-575c	single[b]	(22B)[a]	single[b]
THL-479d	VC I			TG-437b	VC XIV
THL-459d	VC I				
THL-78					
(32B)[a]	single[b]				
THR-688f	single[b]				
THR-636b	VC II				
THR-581a	VC II				
THR-510c	VC II				
THR-454e	VC II				
THR-402f	VC XVI				
14 isolates		4 isolates		5 isolates	
6 VC groups		4 VC groups		5 VC groups	

[a] Isolate collected in 1997- 1998 and renamed with updated nomenclature.
[b] Isolate belongs to a vegetative compatibility group composed of only itself.

Table 4.6. White *Cryphonectria parasitica* isolates recovered from cankers on the three grafted American chestnut trees classified with CM group 3 cultural characteristics (white or slightly pigmented centers with numerous lightly pigmented yellow-orange pycnidia on margins) at 14 days on single spore plates

CM group 3

TH graft		**RM graft**		**TG graft**	
Isolate	VC group	Isolate	VC group	Isolate	VC group
THL-390a	single[b]	RML-785f	VC IV	TG-659b	VC XIX
THL-335d	VC XVIII	RML-620b	VC V	TG-568a	VC XI
THL-335f	VC XVIII	RML-620c	VC V	TG-568e	VC XI
THL-230b	single[b]	RML-620e	VC V	TG-568f	VC XI
THR-636d	VC II	RML-620f	VC V	TG-551c	VC XII
THR-340f	VC XVII	RML-610e	VC XVII	TG-551e	VC XII
THR-270d	VC IX	RML-610f	VC XVII	TG-551f	VC XII
THR-124		RML-370b	VC XXII	TG-539d	VC XIII
(33D)[a]	single[b]	RML-370d	VC XXII	TG-539e	VC XIII
		RML-88		TG-539f	VC XIII
		(2B)[a]	single[b]	TG-437c	VC XIV
		RMR-575e	VC X	TG-217	
		RMR-575f	single[b]	(27A)[a]	VC XIX
		RMR-494f	VC XXI	TG-174	
		RMR-275		(23D)[a]	VC IV
		(17A)[a]	VC XXV	TG-132	
				(26A)[a]	single[b]
				47A[c]	VC III
8 isolates		14 isolates		15 isolates	
7 VC groups		8 VC groups		8 VC groups	

[a] Isolate collected in 1997 - 1998 and renamed with updated nomenclature.
[b] Isolate belongs to a vegetative compatibility group composed of only itself.
[c] Isolate from 1997 – 1998 that could not be identified to exact position on the grafted tree.

Table 4.7. White *Cryphonectria parasitica* isolates recovered from cankers on the three grafted American chestnut trees classified with CM group 4 cultural characteristics (lightly yellow-orange pigmented centers with white "fast, advancing " margins) at 14 days on single spore plates

CM group 4

TH graft		**RM graft**		**TG graft**	
Isolate	VC group	Isolate	VC group	Isolate	VC group
THL-479e	VC I	RML-610d	single[b]	TG-470f	VC XVII
THL-230f	VC XVI	RML-525c	single[b]	TG-388(25)[a]	VC XV
THL-222 (36D)[a]	single[b]	RML-508c	VC VI	TG-287b	VC XV
		RML-285c	VC XX		
THR-402e	VC XVI	RML-285b	VC XXIII		
THR-270d	single[b]	RML-160 (5B)[a]	single[b]		
THR-129 (33A)[a]	VC XVI				
6 isolates		6 isolates		3 isolates	
4 VC groups		6 VC groups		2 VC groups	

[a] Isolate collected in 1997- 1998 and renamed with updated nomenclature.
[b] Isolate belongs to a vegetative compatibility group composed of only itself.

4.4 Overall and within canker spatial patterns of white and pigmented *C. parasitica* isolates and VC groups

Spatial patterns of the white isolates and of the white VC groups among the 49 cankers, on the three grafts, were found to be random and aggregated, respectively (P= 0.325 and P= 0.019) using the double matrix test of Harvey et al. (1988) (Table 4.8). All isolates of *C. parasitica* (both white and pigmented) recovered from all 49 cankers also had a random pattern (P=0.840). In addition, the Lloyd's index of patchiness value for the white isolates in cankers on all three trees was 0.91. This value is just less than 1.0, which would indicate a random pattern (Pielou 1977).

Spatial patterns of white and pigmented isolates within lattice plots were determined for six cankers from the chestnut grafts. Two cankers were chosen for each tree: one main stem canker and one branch canker. The results obtained for the six cankers are presented in Fig 4.3 – 4.8. The number and percentage of white isolates ranged from 0 (cankers THL-660 and TG-485) to 27 (60%) (Canker THL-450), for the 49 lattice cells in each plot (Table 4.9). Frequently a white and pigmented isolate were recovered from the same bark core (12 cores). In these cases, each isolate was used in spatial pattern determination. Spatial pattern determination of the white isolates in these cankers, using the join-count statistics described by Pielou (1977), indicated that three of the four cankers (TG-303, RMR-470, THL-450 and RML-470) containing white isolates had random patterns (Table 4.10). The forth canker (THL-450) had an aggregated pattern of white isolates.

Vegetative compatibility tests on both white and pigmented isolates were performed for branch canker, TG-303, and main stem canker, RML-470. Canker RML-470 had a total of nine VC groups with two groups (A and B) consisting of both white and

pigmented isolates. Vegetative compatibility group A was the predominant VC group, containing 18 white and 12 pigmented isolates (Table 4.10, Fig 4.9 - 4.10). Each of the lattice cells yielding white or pigmented isolates within VC group A were either in contact with each other or were separated by only one lattice cell. This group was not compatible with any of the 25 major VC groups established in this study. The other seven VC groups were composed of exclusively white or pigmented isolates. Spatial pattern determination indicated that VC group A (containing white and pigmented isolates) had a random pattern within the canker (Table 4.10)

The findings from branch canker TG-303 were similar to those of canker RML-470. Canker TG-303 had a total of 10 VC groups, seven of which were composed of single white or pigmented isolates (Fig 4.11 – 4.12). There was one predominant group (AA) that contained a total of nine white and 17 pigmented isolates, and was found to be compatible with VC group XV from the 25 major VC groups. With one exception, the lattice cells yielding white or pigmented isolates in VC group AA were in contact with each other or were separated by only one lattice cell. The spatial pattern of the white isolates and VC group XV (AA) (containing white and pigmented isolates) in canker TG-303 were both found to be random at the 5% significance, using the join-count statistics described by Pielou (1977) (Table 4.10).

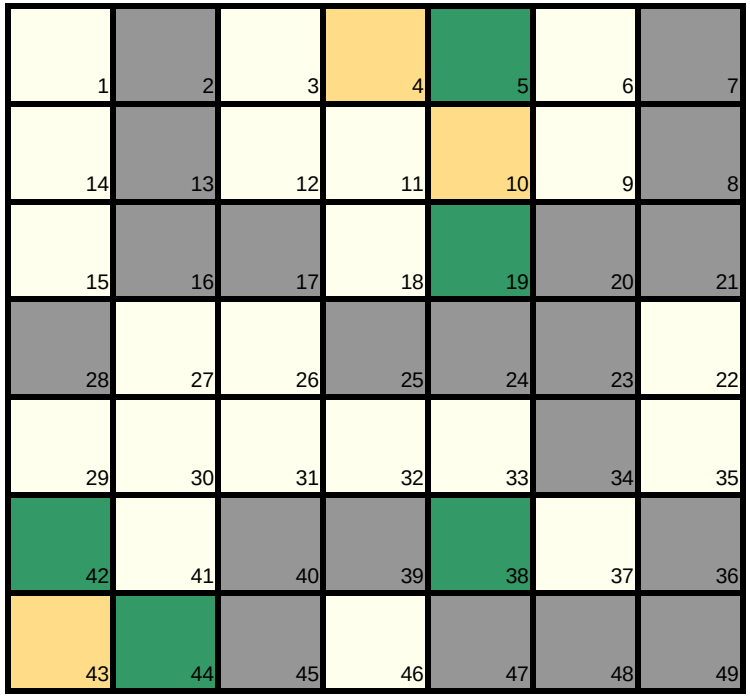
Table 4.8. Spatial pattern analysis for culture types (white alone/or white and pigmented) and white vegetative compatibility (VC) groups of *Cryphonectria parasitica* isolates recovered from 49 cankers on grafted American chestnut trees

Category	N[a]	V_2[b]	G/V_2[c]	$G_{(p)}$[d]	P[e]
White isolates	110	1	29.37	29.90	0.325
White and pigmented isolates	217	2	10.96	10.27	0.840
White VC groups	110	25	1285.7	1925.4	0.019

[a]Number of isolates included in the analysis.
[b]Number of culture types or VC groups that occurred more than once.
[c]Observed mean distance (in cm) of isolates with the same culture type or VC group.
[d]Expected mean distance (in cm) under the assumption that culture types or VC groups are assigned at random.
[e]Probability of the observed mean distance (G/V_2) compared with the mean distance ($\Gamma_{(p)}$) that was expected after 1,000 randomizations under the null hypothesis of randomness.

Stem Canker RML-470

Pattern of White Isolates



Pattern of Pigmented Isolates

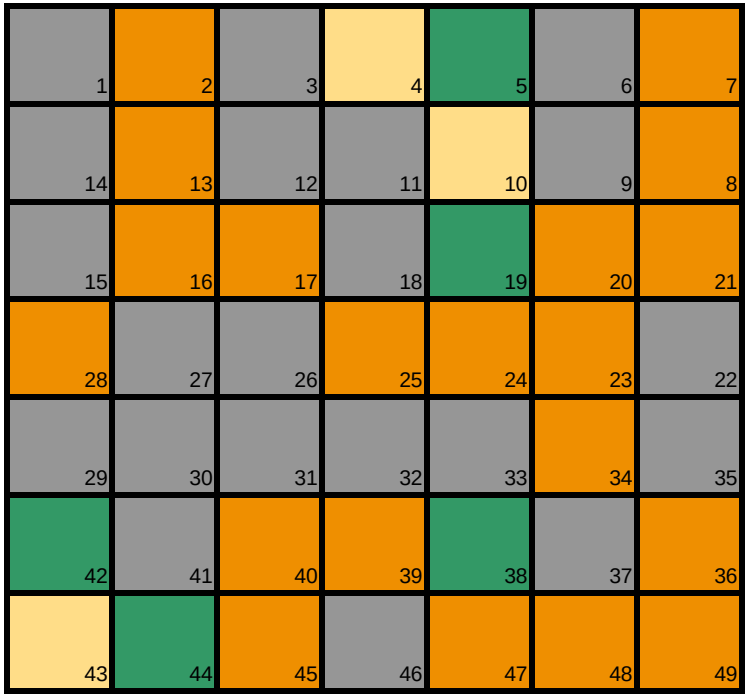
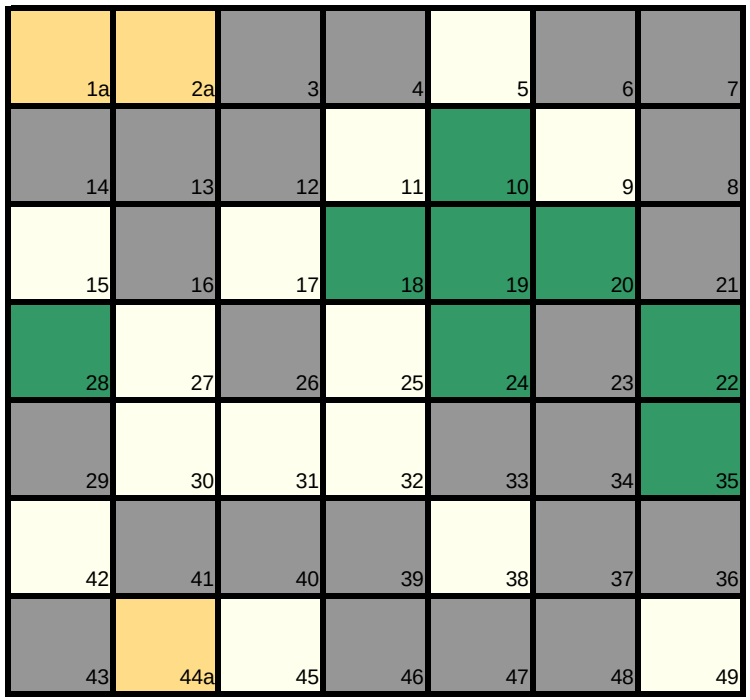


Fig 4.3. Pattern of white and pigmented isolates of *Cryphonectria parasitica* in a lattice plot (17.8 x 17.8 cm) of stem canker RML-470. White and orange boxes represent lattice cell that yielded white and pigmented isolates, respectively. Striped boxes represent cells that yielded both white and pigmented isolates. Isolates recovered that were other than *C. parasitica* are portrayed by green boxes

Branch Canker TG-303

Pattern of White Isolates



Pattern of Pigmented Isolates

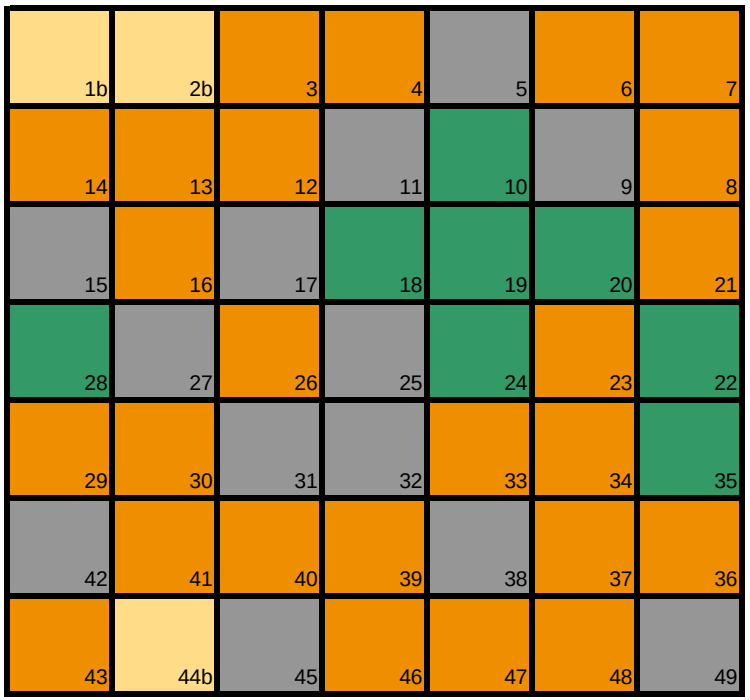
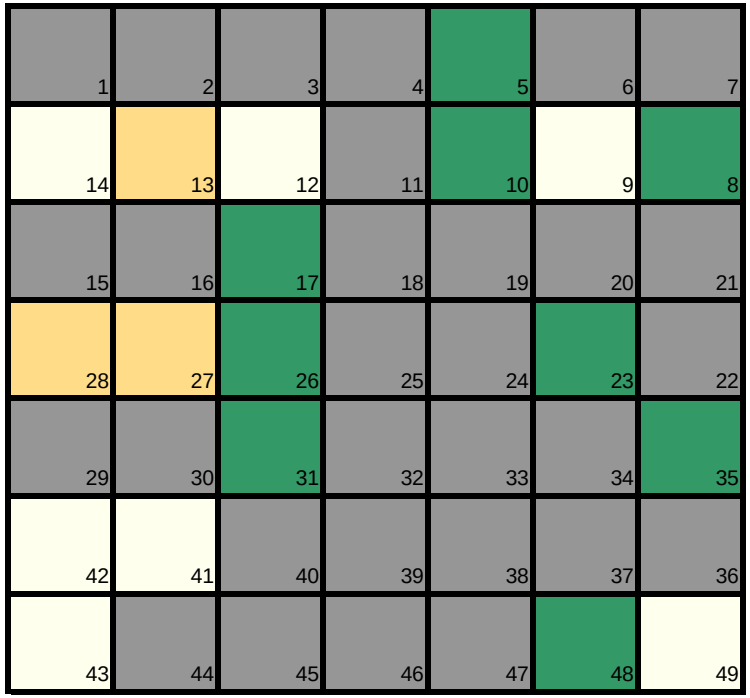


Fig 4.4 . Pattern of white and pigmented isolates of *Cryphonectria parasitica* in a lattice plot (17.8 x 17.8 cm) of branch canker TG-303. White and orange boxes represent lattice cell that yielded white and pigmented isolates, respectively. Striped boxes represent cells that yielded both white and pigmented isolates. Isolates recovered that were other than *C. parasitica* are portrayed by green boxes

Branch Canker RMR-470

Pattern of White Isolates



Pattern of Pigmented Isolates

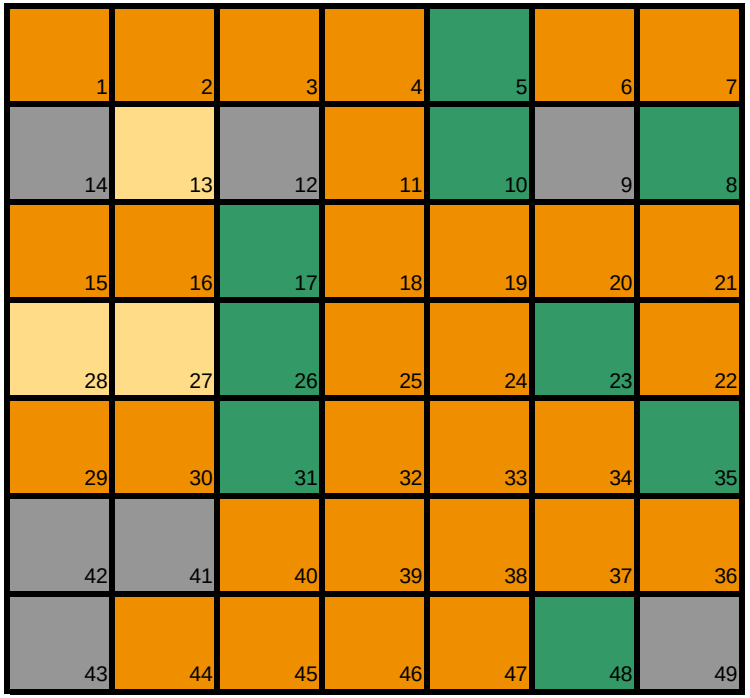
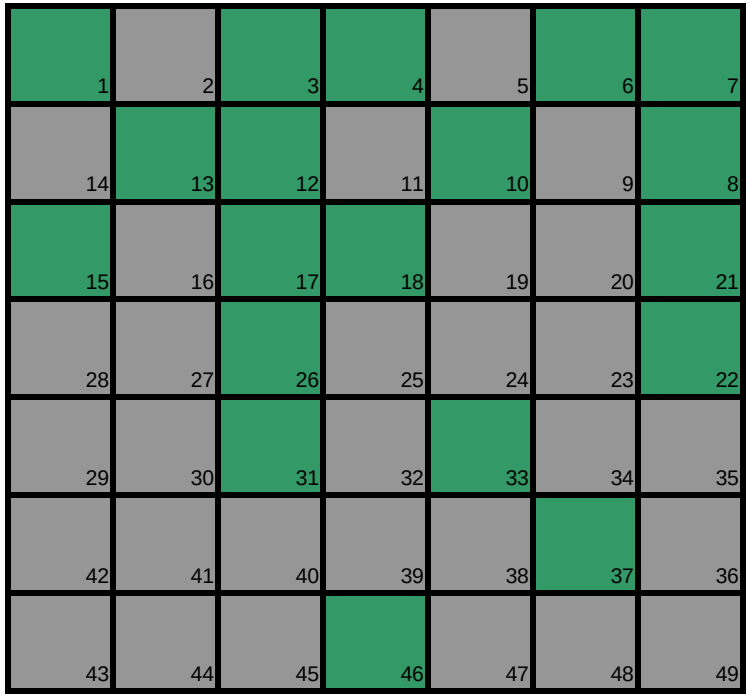


Fig 4.5 . Pattern of white and pigmented isolates of *Cryphonectria parasitica* in a lattice plot (17.8 x 17.8 cm) of branch canker RMR-470. White and orange boxes represent lattice cell that yielded white and pigmented isolates, respectively. Striped boxes represent cells that yielded both white and pigmented isolates. Isolates recovered that were other than *C. parasitica* are portrayed by green boxes

Stem Canker TG-485

Pattern of White Isolates



Pattern of Pigmented Isolates

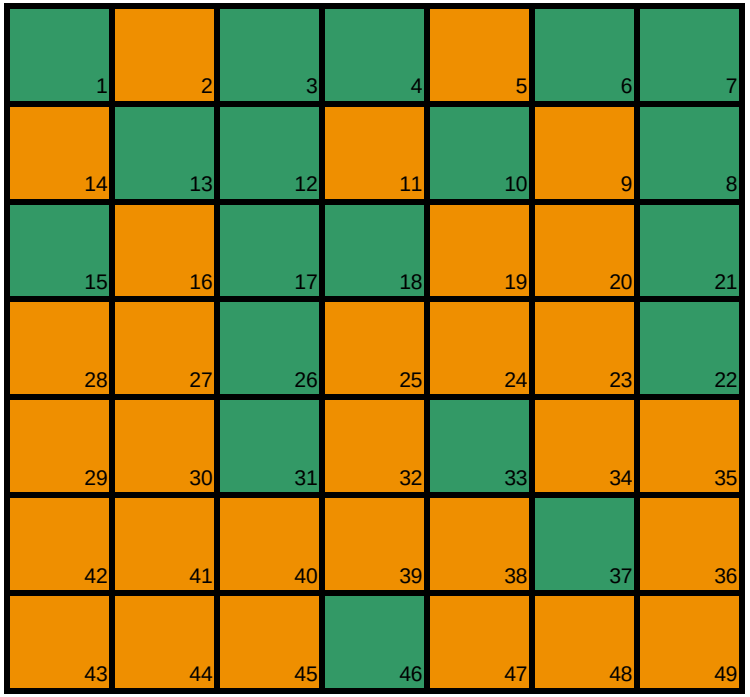
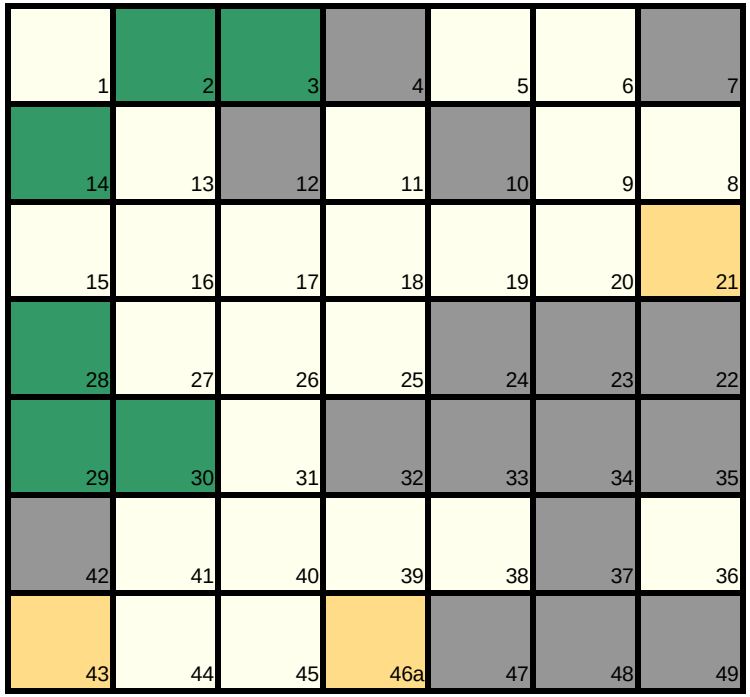


Fig 4.6. Pattern of white and pigmented isolates of *Cryphonectria parasitica* in a lattice plot (17.8 x 17.8 cm) of stem canker TG-485. White and orange boxes represent lattice cell that yielded white and pigmented isolates, respectively. Striped boxes represent cells that yielded both white and pigmented isolates. Isolates recovered that were other than *C. parasitica* are portrayed by green boxes

Stem Canker THL-450

Pattern of White Isolates



Pattern of Pigmented Isolates

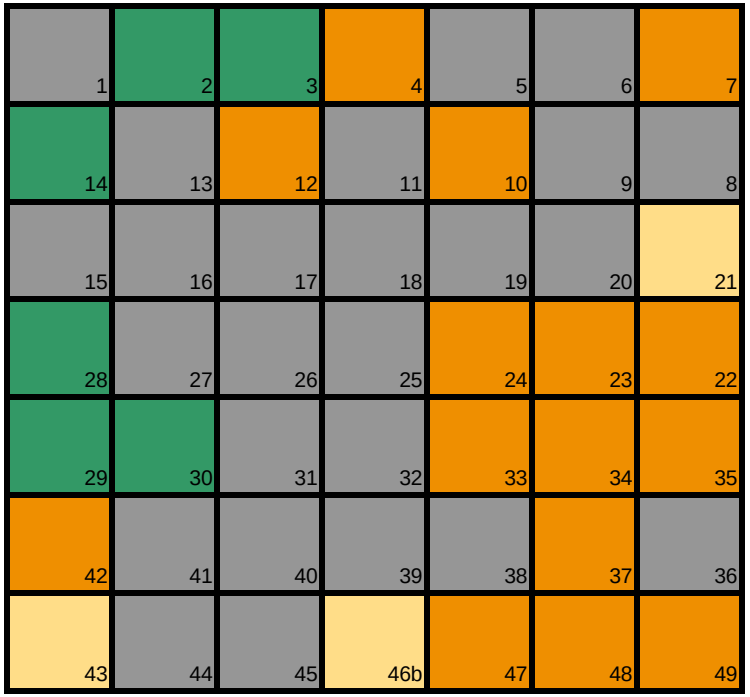
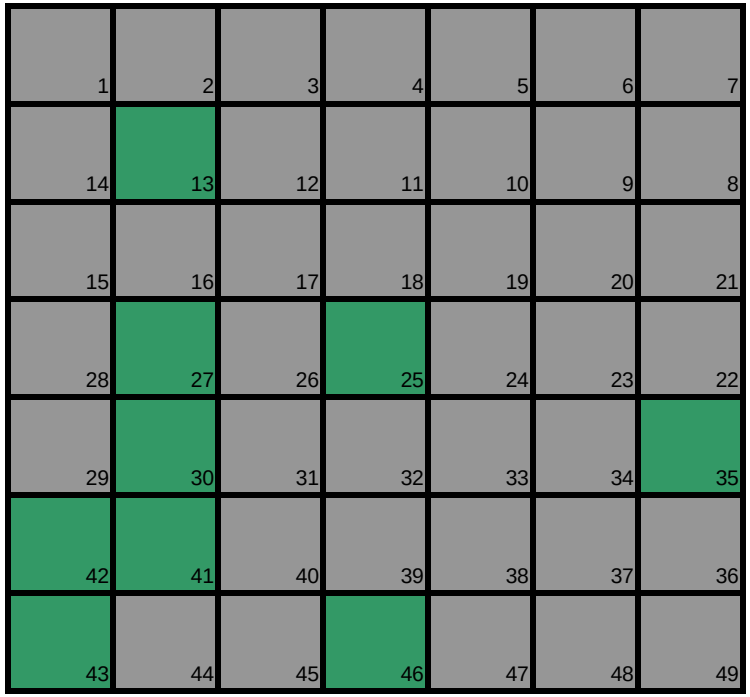


Fig 4.7. Pattern of white and pigmented isolates of *Cryphonectria parasitica* in a lattice plot (17.8 x 17.8 cm) of stem canker THL-450. White and orange boxes represent lattice cell that yielded white and pigmented isolates, respectively. Striped boxes represent cells that yielded both white and pigmented isolates. Isolates recovered that were other than *C. parasitica* are portrayed by green boxes

Branch Canker THR-660

Pattern of White Isolates



Pattern of Pigmented Isolates

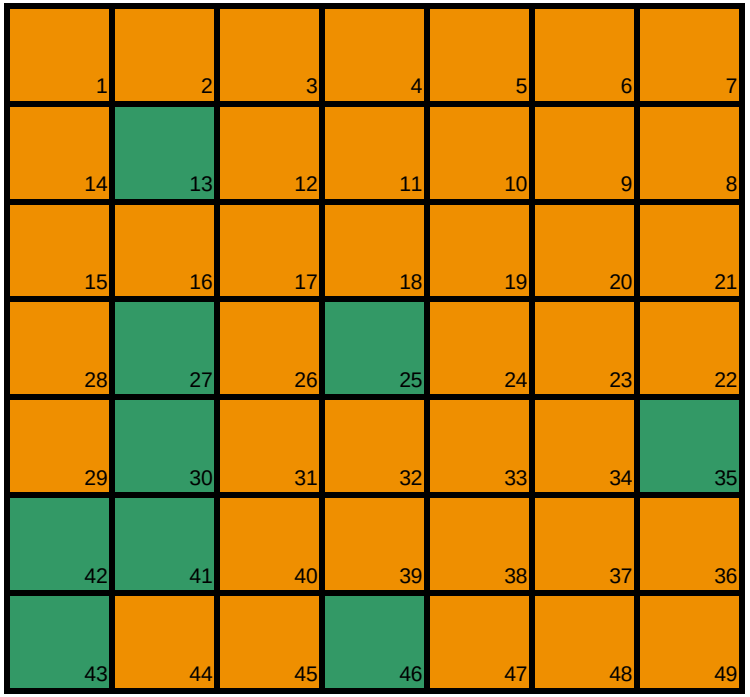


Fig 4.8. Pattern of white and pigmented isolates of *Cryphonectria parasitica* in a lattice plot (17.8 x 17.8 cm) of branch canker THR-660. White and orange boxes represent lattice cell that yielded white and pigmented isolates, respectively. Striped boxes represent cells that yielded both white and pigmented isolates. Isolates recovered that were other than *C. parasitica* are portrayed by green boxes

Stem Canker RML-470

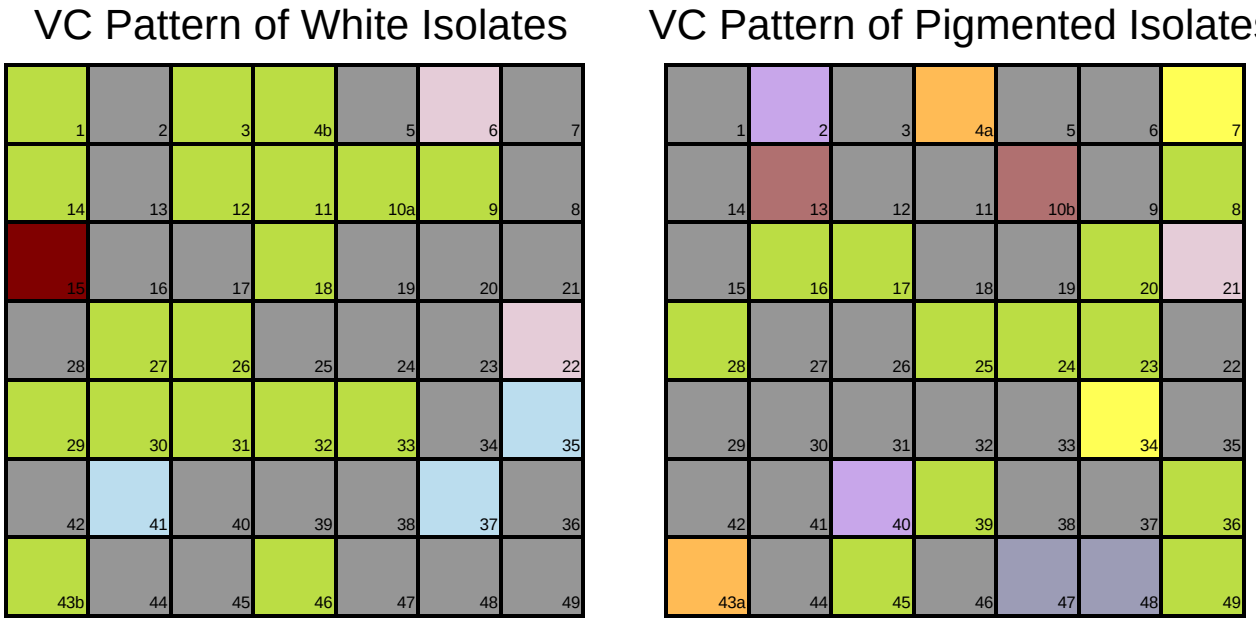
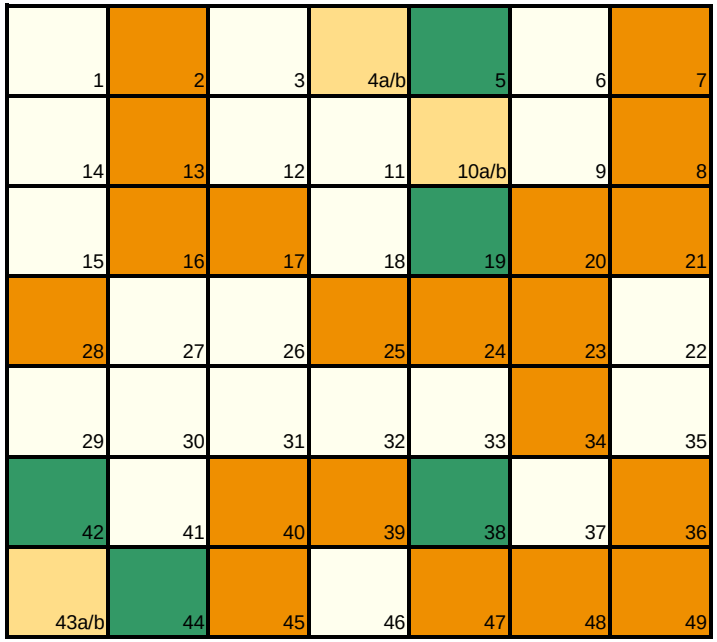


Fig 4.9. Pattern of white and pigmented vegetative compatibility (VC) groups of *Cryphonectria parasitica* in a 7 x 7 lattice plot (17.8x 17.8 cm) of stem canker RML-470. There are a total of nine VC groups composed of of 47 *C. parasitica* isolates. VC group A is the largest group, representing 30 white and pigmented isolates

VC group A: 30 isolates
VC group B: 3 isolates
VC group C: 3 isolates
VC group D: 2 isolates
VC group E: 2 isolates
VC group F: 2 isolates
VC group G: 2 isolates
VC group H: 2 isolates
VC group I: 1 isolate

Stem Canker RML-470

A. Overall pattern of pigmented and white isolates



B. Pattern of pigmented and white isolates in the same vc group

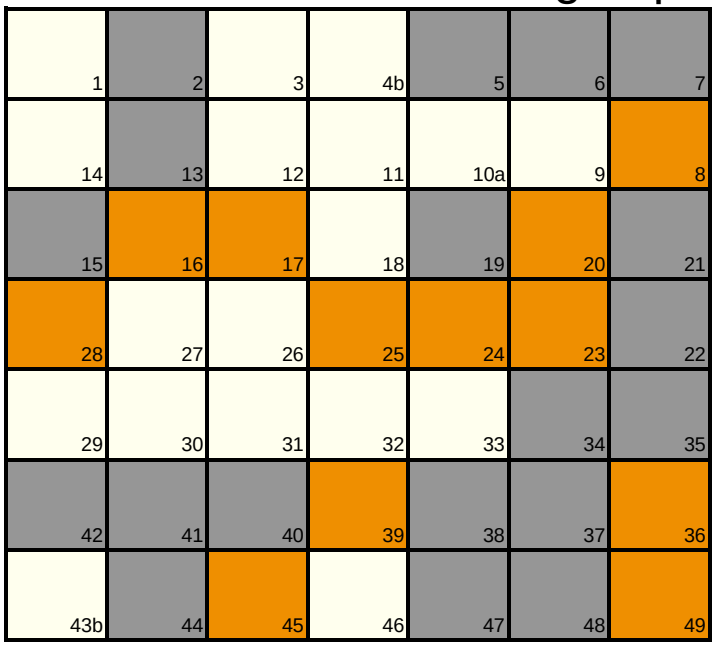
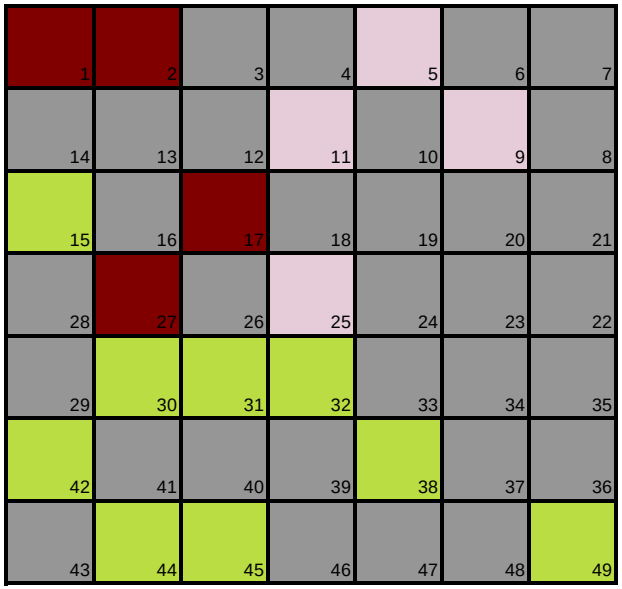


Fig 4.10 . A) Pattern of white and pigmented isolates of *Cryphonectria parasitica* in a lattice plot (17.8x 17.8 cm) of stem canker RML-470. White and orange boxes represent lattice cell that yielded white and pigmented isolates, respectively, and striped boxes represent cells that yielded both white and pigmented isolates. Fungal isolates recovered that were other than *C. parasitica* are portrayed by green boxes. B) Distribution of white and pigmented isolates in the same major vc group (A). There are 29 joins among white and pigmented lattice cells representing 29 possible connection points for hypovirulence conversion. Gray lattice cells yielded other fungal isolates

Branch Canker TG-303

VC Pattern of White Isolates



VC Pattern of Pigmented Isolates

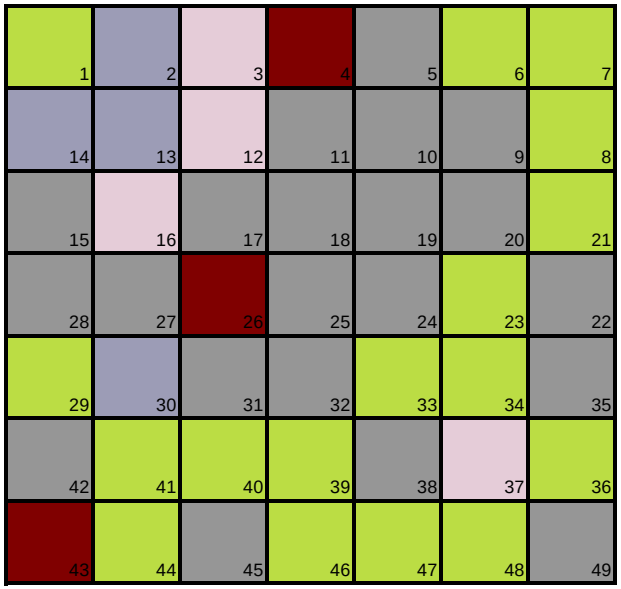


Fig 4.11 . Pattern of white and pigmented vegetative compatibility (VC) groups of *Cryphonectria parasitica* in a 7 x 7 lattice plot (17.8x 17.8cm) of branch canker TG-303. There are three main groups and seven single VC groups (containing one isolate); yielding a total of 10 vegetative compatibility groups in the TG-303 branch canker

VC group AA: 26 isolates

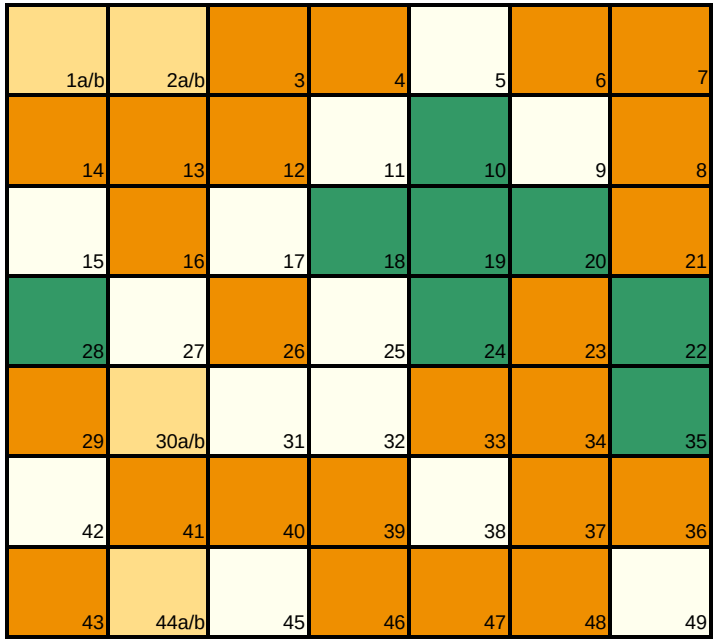
VC group BB: 8 isolates

VC group CC: 4 isolates

single isolate VC groups: 7

Branch Canker TG-303

A. Overall pattern of pigmented and white isolates



B. Pattern of pigmented and white isolates in the same vc group

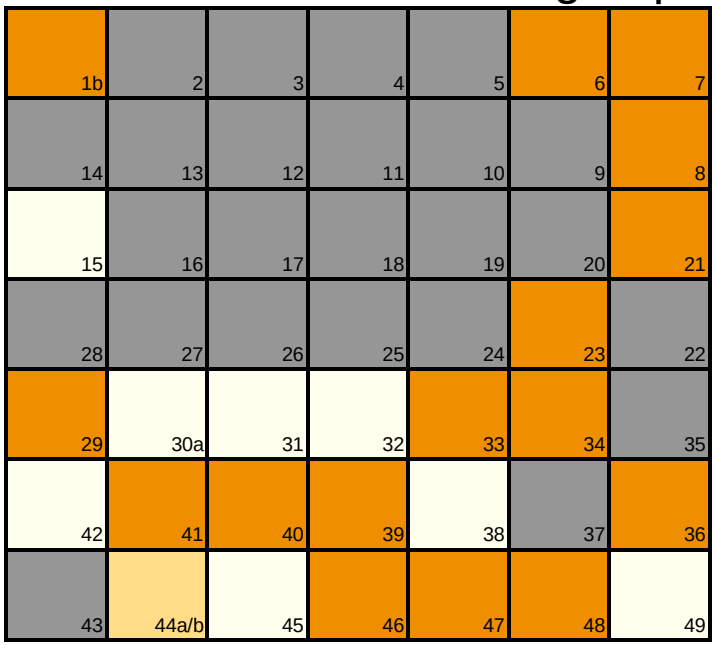


Fig 4.12 . A) Pattern of white and pigmented isolates of *Cryphonectria parasitica* in a lattice plot (17.8x 17.8 cm) of branch canker TG-303. White and orange boxes represent lattice cells that yielded white and pigmented isolates respectively, and striped boxes represent cells that yielded both white and pigmented isolates. Fungal isolates recovered that were other than *C. parasitica* are portrayed by green boxes. B) Distribution of white and pigmented isolates in the same major VC group (AA). There are 28 joins among white and pigmented lattice cells representing 28 possible connection points for hypovirulence conversion. Gray lattice cells yielded other fungal isolates

Table 4.9. Number of white and pigmented *Cryphonectria parasitica* isolates collected from main stem and branch cankers using a 49-cell lattice plot (17.8 x 17.8 cm)

	Canker	No. White isolates	No. Pigmented isolates	% White isolates[a]	% Lattice cells yielding white isolates[b]
Main stem cankers	THL-450[c]	27	18	60.0	55.1
	RML-470	24	22	52.2	49.0
	TG-485	0	39	0	0
Branch cankers	THR-660[c] (513 +157 cm)	0	40	0	0
	RMR-470 (440 + 30 cm)	10	33	23.3	20.4
	TG-303 (70 + 233 cm)	17	28	37.8	34.7

[a] Percentage is calculated from total *C. parasitica* isolates collected.
[b] Percentage is calculated from total number of lattice cells (49).
[c] Main stem cankers are measured from the ground to center of canker. Branch cankers are measured from the ground to center of branch and then to center of canker.

Table 4.10. Frequency and spatial statistics for major VC groups (includes white plus pigmented isolate) and white *Cryphonectria parasitica* isolates recovered from 7 x 7 lattice plots of cankers on TH, RM, and TG grafted American chestnut trees

Canker code	Number of isolates recovered per lattice cell[a]			Major VC group[b]	W spatial statistics[c]		VC spatial statistics[c]	
	W	P	W/P		Z statistic	p-value	Z statistic	p-value
RML-470	24	23	3	A	-0.145	0.44	1.357	0.088
TG-303	17	28	3	AA	-0.314	0.38	0.762	0.223
THL-450	27	18	3		2.113*	0.017*		
RMR-470	10	33	3		0.015	0.49		
THL-660	0	40	0		—	—		
TG-480	0	30	0		—	—		
Mean	13	28.7	2					

[a]Numbers of white (W) and pigmented (P) isolates recovered from 49 lattice cells in a 7 x 7 lattice plot attached to each canker. W/P represents the number of lattice cells that yielded both white and pigmented isolates.
[b]Major vc group consisting of white and pigmented isolates identified to vc group for RML-470 and TG-303, respectively.
[c] Z statistic and corresponding p-value obtained in tests of significance for the null hypothesis of random mingling of white isolates or major vc groups includes white plus pigmented isolates in each lattice plot. The null hypothesis was rejected (*) only for pattern of white isolates in canker THL-450 at $Z \geq 1.96$.

4.5 dsRNA assays of pigmented isolates recovered from cankers used in spatial pattern studies

Several pigmented isolates from spatial pattern cankers TG-303 and THL-660 were examined for the presence of dsRNA. Canker THL-660 yielded no white isolates; however, the canker was classified as superficial in the field. It was hypothesized that these pigmented isolates could be hypovirulent and contain dsRNA. Canker TG-303 contained both pigmented and white isolates in the same VC group. It was hypothesized that these pigmented isolates might also contain dsRNA. Six isolates were randomly chosen from the THL-660 canker and five isolates from the TG-303 canker. Each isolate was tested for the presence of dsRNA. All six isolates from THL-660 and all five isolates from TG-303 were negative for the presence of dsRNA.