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The Effect of Warming Winter Temperatures on the Severity of Pierce's Disease in the Appalachian Mountains and Piedmont of the Southeastern United States

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Abstract
Pierce's disease (PD) caused by *Xylella fastidiosa* (*Xf*) is a major threat to the rapidly growing *Vitis vinifera*/French-American hybrid winegrape industry in the southeastern United States. The bacterium, which is transmitted by xylem-feeding insects, is unable to survive low winter temperatures, and infected vines often recover the next year. Maps available from the 1970s indicate that PD is not a serious threat in the southeastern US, where the average minimum January temperature is ≤ 1.7°C. However PD symptoms developed in many vineyards planted in the late 1990s in areas identified as low risk. Surveys conducted in North Carolina and Georgia confirmed the presence of *Xf* in symptomatic vines using ELISA kits. Weather data for November to March from 84 weather stations from 1972-2005 for Alabama, Georgia, North Carolina, South Carolina, Tennessee, and Virginia were used to construct new maps using ArcGIS 9.1, and PD survey data from vineyards were superimposed on the maps. Areas for low risk for PD corresponded most closely with a minimum winter temperature of ≤ -12.2°C for 2 to 3 days or ≤ -9.4°C for 4 to 5 days. Warm winter temperatures during the last 8 years have resulted in a significant shift in the isotherms towards the north and west, increasing the risk of PD in the Piedmont region of the Southeast, and have extended the threat into Virginia and Tennessee.

Introduction
The *Vitis vinifera*/French American hybrid winegrape industry has experienced rapid growth in North Carolina as well as other southeastern states over the past 10 years, but further expansion of the industry may be limited by Pierce's disease (PD). PD is caused by *Xylella fastidiosa* Wells et al. (*Xf*) (19), a gram-negative fastidious xylem inhabiting bacterium which is spread from plant-to-plant by xylem feeding insects with piercing mouth parts such as sharpshooters, leafhoppers, and spittle-bugs (15). *Xf* is geographically limited to areas with mild winters where PD can be the principal limiting factor in the production of *vinifera*/French American hybrids and *Vitis labrusca* (10). In areas with cold winters and freezing temperatures, infected vines have been known to recover the following spring (7,16), because populations of *Xf* within the grape xylem tissue decline under cold conditions (6). This "cold curing" is related to biochemical changes in vines exposed to cold temperatures (12). In North Carolina, the disease is well documented in the milder Coastal Plain region on *V. labrusca* and *V. rotundifolia* (14). Currently available maps [(9), UC Berkeley *X. fastidiosa* website, www.cnr.berkeley.edu/xylella/overview/page2.html] based primarily on anecdotal information, indicate that the disease is more sporadic or not a serious threat in the cooler Piedmont region. The map on the UC Berkley *Xylella fastidiosa* website defines areas in North Carolina, South Carolina, Georgia, and Alabama, where the disease is rare, by an isotherm based on an average minimum January temperature of ≤ 1.7°C (Fig. 1). Most of the expansion of the industry in the Southeast has been in the Piedmont and Mountain regions of the states inside this isotherm or "blue" zone (Fig. 1). However, soon after vineyards were planted in this zone, vines symptomatic of Pierce's disease were observed in some

vineyards. Because of the high cost of establishing a vineyard, it is essential to warn growers not to plant in areas where the likelihood of PD is great. Thus the goal of this research was: (i) to conduct surveys to confirm the presence of PD in newly planted vineyards in the Piedmont and Mountain regions of North Carolina and Georgia; (ii) to relate the presence of PD to winter temperatures within the regions; and (iii) to identify and map low, moderate, and high-risk areas for PD in the Southeast. These maps should be useful to growers as a guide for making planting decisions in the southeastern United States; and may also help to explain the spread of the disease into areas which had been believed to be disease free.

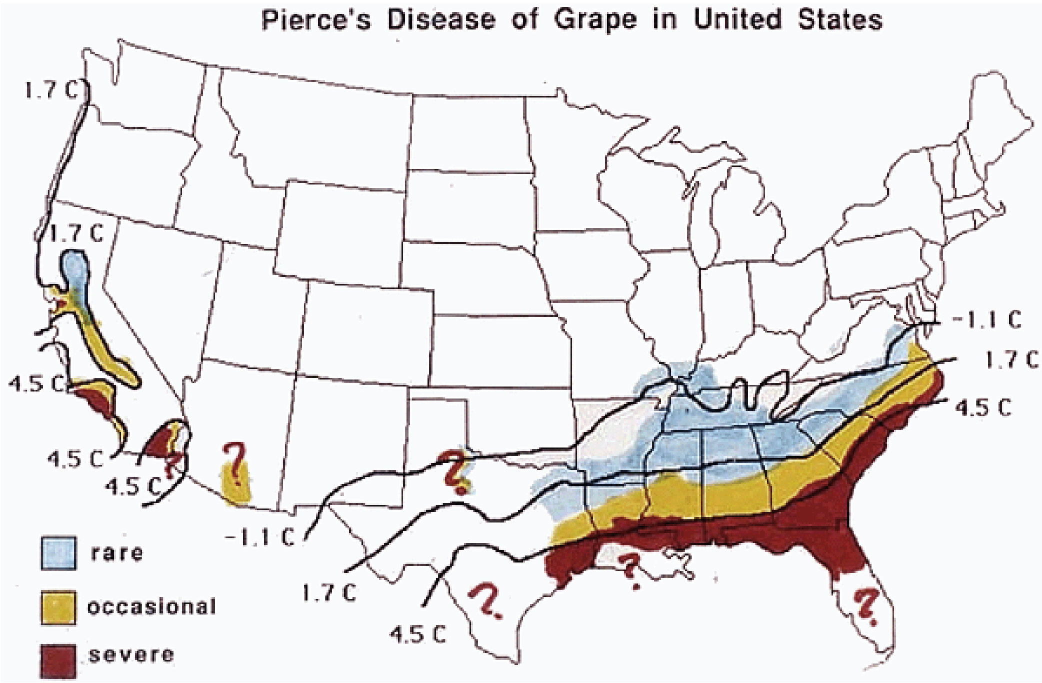


Fig. 1. Map of United States showing probable occurrence of Pierce’s disease. From UC Berkeley *X. fastidiosa* website.

Surveys for Pierce’s Disease

In late summer of 2001, 22 vineyards of *V. vinifera* and French-American hybrid grapes were selected arbitrarily within the winegrape growing region of the Piedmont and Mountains of North Carolina. Vines were scouted in each vineyard for three characteristic symptoms of PD: leaf scorch showing marginal chlorosis and necrosis; leafless petioles (match-sticks); and "green islands" on the canes (Figs. 2 A, B, and C). Symptomatic leaves or leafless petioles which exhibited the typical "match-stick" symptom for PD were collected from symptomatic vines. One to four samples were tested for *Xf* according to procedures described for Agdia DAS ELISA test kits (Agdia Incorporated, Elkhart, IN) (Table 1). In 2002, 14 additional vineyards were scouted and symptomatic vines tested for *Xf* as described above. One additional vineyard was sampled in both 2003 and 2004 and is included in the survey results (Table 1). Between 2000 and 2006, eight vineyards where symptomatic vines were observed were surveyed in north Georgia. Samples were tested for *Xf* as described above and were confirmed through isolation on PW medium. Vineyards which tested positive for *Xf* had a range of symptoms from one or two leaves showing marginal chlorosis to complete vines showing "match-stick" symptoms.

Fig. 2. Pierce’s disease symptoms on grape vines showing (**A**) marginal chlorosis and necrosis, (**B**) leafless petioles "match-stick" symptoms, and (**C**) "green island" symptoms.

Table 1. Vineyards and their locations surveyed for Pierce's disease from 2001 to 2005.

Vineyards surveyed	**Location** (deg min sec)		**Elev-ation** (m)	**Year tested**	**ELISA results**
	Longitude	**Latitude**			
Biltmore, NC	82 34 51.55W	35 32 28.57N	641	2001	+
Black Wolf, NC	80 42 09.19W	36 24 19.44N	338	2001	+
Capozzi, NC	82 01 08.75W	35 15 53.41N	250	2002	+
Cerminaro, NC	81 18 46.73W	36 02 10.81N	381	2002	+
Chinaquapin, NC	79 39 36.00W	36 22 12.01N	232	2001	+
Cloer's, NC[x]	78 55 44.43W	35 45 58.78N	98	2004	+
Creek Side Winery, NC	79 29 20.24W	36 13 36.55N	162	2002	+
Dennis, NC	80 16 54.82W	35 18 37.21N	207	2001	+
Gray Draughn, NC	80 36 08.53W	36 23 10.08N	350	2002	+
Hanover Park, NC[x]	80 37 26.17W	36 03 44.56N	278	2001	+
Hinnant, NC	78 14 01.34W	35 31 20.68N	46	2001	+
Iron Gate, NC[x]	79 16 03.86W	36 09 21.57N	203	2003	+
Jeff Bloodworth, NC	79 04 12.74W	36 06 41.95N	178	2001	+
Laurel Gray, NC	80 49 58.18W	36 08 00.73N	346	2002	+
Lawrence, NC	79 57 01.00W	36 18 00.01N	259	2001	+
Lenox Castle, NC	79 33 19.95W	36 17 41.70N	230	2001	+
Marvin Pack, NC	82 06 31.63W	35 14 23.19N	326	2002	+
Mize, NC[x]	82 01 08.22W	35 13 04.84N	307	2001	+
Raffaldini, NC	80 53 00.09W	36 10 46.67N	337	2001	+
RagApple Lassie, NC[x]	80 39 12.67W	36 13 09.65N	335	2001	+
Raylen, NC	80 29 54.23W	35 58 07.88N	255	2002	+
Reidsville Research Station, NC[x]	79 41 05.09W	36 22 31.33N	217	2002	+
Rockhouse, NC[x]	82 05 34.37W	35 16 25.16N	317	2001	+
Round Peak, NC[x]	80 47 12.94W	36 30 46.83N	390	2002	+
Shelton, NC	80 45 34.78W	36 20 51.23N	378	2002	+
Silk Hope, NC[x]	79 16 47.45W	35 46 04.05N	223	2001	+
Silver Creek Winery, NC	80 42 12.20W	35 43 51.49N	240	2002	+
Slater, NC	82 05 15.68W	35 11 31.55N	266	2001	+
Summer Duck, NC	79 51 18.57W	35 07 58.18N	148	2001	+
Westbend, NC[x]	80 30 00.96W	36 05 22.61N	246	2001	+
Windy Gap, NC	80 56 55.36W	36 09 25.63N	353	2002	+
Hincher, NC	80 54 07.42W	36 09 59.62N	444	2001	—
Jerry Haynes, NC	80 51 59.14W	36 20 30.64N	400	2001	—
Maple Dr. Asheville, NC	82 29 56.60W	35 36 37.85N	680	2001	—
Ratliff, NC	82 19 56.79W	35 14 38.75N	636	2002	—
Ritler Ridge, NC	82 42 00.00W	35 32 24.01N	657	2001	—
Silo House, NC	82 59 28.38W	35 29 38.04N	846	2002	—
Stepp, NC	82 22 12.00W	35 22 22.48N	662	2001	—
Braselton, GA	83 49 01.15W	34 05 55.64N	280	2000-6	+
Dahlonega, GA	83 50 05.56W	34 30 58.35N	526	2004-5	+
Ila, GA	83 17 25.28W	34 11 04.98N	240	2004-5	+
Ocilla, GA	83 16 30.89W	31 33 51.42N	102	2004-5	+
Social Circle, GA	83 43 34.88W	33 38 07.20N	261	2004-5	+
Tiger, GA	83 25 57.22W	34 51 22.77N	596	2004-6	±[y]
Blairsville, GA	83 52 56.57W	34 57 51.53N	576	2004-5	—
Clayton, GA	83 24 23.57W	34 52 57.25N	599	2004-5	—

[x] Detail survey of selected varieties was conducted and individual vines were rated on a scale of 1 to 4 for PD.

[y] One confirmed vine with PD found in 2006.

Based on the symptoms observed, a rating system for scoring individual vines was developed where 0 = no leaves showing any symptoms; 1 = one shoot with one or more leaves showing marginal chlorosis or necrosis; 2 = two to three shoots with one or more leaves showing PD symptoms; 3 = multiple affected shoots; 4 = entire plant showing PD symptoms with shriveled clusters and many leafless petioles; and dead = vine dead wood with no leaves. Over the course of this study and others (*not reported*) we evaluated our rating system on 261 vines: 15.8% of vines rated as 0 gave positive ELISA results; 40.6% of those rated 1; 52.5% of those rated 2; and 81.1% of those rated 3 were positive. These results are similar to those of Krell et al. 2006 who found that leaf scorch symptoms were not always a good indicator of the presence of *Xf* (13). Krell and others (2,13,17) also showed that *Xf* can be detected in asymptomatic leaves. Petioles with detached leaf blades were much easier to find on vines that scored 3, which may explain the higher percentage of positive results on those vines.

Based on this rating system, a more extensive survey was conducted in different variety blocks in four vineyards (Hanover Park, Rockhouse, Silk Hope, and Westbend) in 2001 (Table 2). In 2002 three vineyards that were surveyed in detail in 2001 (Hanover Park, Silk Hope, and Westbend) were resurveyed and two additional vineyards (RagApple Lassie, and Round Peak) were surveyed. In 2003 detailed surveys were conducted in two additional vineyards (Iron Gate and a vineyard at the Upper Piedmont Research Station in Reidsville, NC) and three previously surveyed vineyards (RagApple Lassie, Silk Hope, and Westbend). In the 2004 a new vineyard was surveyed (Cloer's) and the Iron Gate vineyard was resurveyed. In 2006 four vineyards (Cloer's, Iron Gate, Rockhouse, and Silk Hope) were resurveyed and an additional vineyard (Mize) was surveyed. In 2007 five vineyards (Cloer's, Iron Gate, Mize, Rockhouse, and Silk Hope) were resurveyed.

Table 2. Detail survey of vineyards in North Carolina. Individual vines were rated for Pierce's disease.

Vineyard	Variety	Year	*n* = no. of vines	Pierce's disease rating (%) 0 / 1	2	3	4	dead
Cloer's	Cabernet Franc	2004	1041	12.1	68.3	14.2	0.9	4.5
		2007	1041	0.5	37.2	36.8	5.9	19.7
	Chambourcin	2004	313	34.2	45.7	18.2	1.0	1.0
		2006*	312	22.4	44.2	19.6	5.1	8.7
	Seyval Blanc	2004	558	0.5	84.6	13.4	0.2	1.3
		2007	550	8.0	66.4	12.2	1.1	12.4
Hanover Park	Cabernet Franc	2001	98	73.5	24.5	1.0	0.0	1.0
		2002*	98	73.5	5.1	1.0	3.1	17.3
	Cabernet Sauvignon	2001	91	84.6	14.3	0.0	0.0	1.1
		2002*	91	62.6	5.5	3.3	1.1	27.5
	Chambourcin	2001	103	38.8	45.6	12.6	2.9	0.0
	Chardonnay	2001	87	56.3	37.9	4.6	0.0	1.1
		2002	87	51.7	31.0	11.5	3.4	2.3
Iron Gate	Chambourcin	2003	279	96.8	0.4	0.0	0.0	2.9
		2004*	280	81.1	17.9	0.0	0.0	1.1
		2007*	280	30.4	63.9	4.6	0.0	1.1
	Merlot	2003	355	89.3	2.8	0.3	0.0	7.6
		2004*	569	10.7	84.9	0.0	0.0	4.4
		2006*	569	28.8	55.4	7.9	0.2	7.7
		2007*	425	7.1	73.4	3.1	0.5	16.0
	Sauvignon Blanc	2004	564	79.6	14.0	0.0	0.0	6.4
Mize	Merlot	2006	430	52.8	14.0	6.5	2.3	24.4
		2007*	430	59.8	12.6	1.4	0.0	26.3
RagApple Lassie	Merlot	2002	432	86.8	13.0	0.0	0.2	0.0
		2003	432	60.0	39.8	0.0	0.0	0.2

(continued)

Table 2 (continued).

Reidsville, NC (UPRS)	Chardonnay	2003	72	91.7	4.2	1.4	0.0	2.8
	Cabernet Franc	2003	192	81.3	5.7	8.9	1.6	2.6
Rockhouse	Cabernet Franc	2001	421	78.9	13.5	3.1	3.8	0.7
		2006*	428	35.7	7.2	8.6	0.2	48.1
		2007*	428	39.5	0.9	0.7	5.4	53.5
Round Peak	Cabernet Franc	2002	358	64.2	20.9	2.8	1.4	10.6
Silk Hope	Cabernet Franc	2001	29	34.5	37.9	17.2	10.3	0.0
		2002*	29	0.0	0.0	69.0	13.8	17.2
		2003*	82	36.6	22.0	7.3	3.7	30.5
		2006*	84	0.0	67.9	10.7	6.0	15.5
		2007*	84	0.0	51.2	21.4	1.2	26.2
	Chambourcin	2001	347	48.4	18.2	11.0	15.6	6.9
		2002*	347	3.2	40.9	19.0	10.4	26.5
		2003*	347	18.4	5.5	6.3	0.6	69.2
		2006*	338	8.9	56.2	12.1	4.1	18.6
		2007*	342	2.9	43.6	31.0	5.0	17.5
	Vidal	2001	87	62.1	13.8	9.2	11.5	3.4
		2002	87	9.2	52.9	18.4	11.5	8.0
		2003*	87	35.6	0.0	1.1	0.0	63.2
		2006	83	12.0	8.4	49.4	7.2	22.9
		2007*	86	7.0	53.5	11.6	3.5	24.4
Westbend	Chambourcin	2001	249	0.8	25.3	57.0	16.1	0.8
		2002	249	18.1	51.0	27.7	2.4	0.8
		2003	250	5.2	51.6	32.4	8.4	2.4
	Chardonnay	2001	314	21.0	42.7	32.2	2.9	1.3
		2002	316	0.0	11.7	59.8	25.9	2.5
		2003*	316	0.6	16.8	35.4	10.1	37.0
	Riesling	2001	310	29.4	25.2	22.3	21.0	2.3
		2002*	310	7.4	20.6	38.4	21.9	11.6
		2003*	310	5.5	24.8	22.3	18.7	28.7

* Dead vines were replaced periodically with replants in the same year or following spring. The percent dead vines in a vineyard over years is not additive, since many growers replanted and replants often did not show symptoms or died during the first year after replanting.

Mapping of Isotherms

Daily temperature data from 1972-2005 from a total of 84 weather stations from Alabama, Georgia, North Carolina, South Carolina, Tennessee, and Virginia were obtained from National Climatic Data Center, www.ncdc.noaa.gov/oa/ncdc.html (Fig. 3). In a preliminary study the number of days with minimum temperatures ranging from ≤ -12.2 to ≤ -6.7°C for November to March for each year was determined for a subset of the weather stations and survey data were positioned on the maps. The accumulated number of days with minimum temperatures either ≤ -12.2 or ≤ -9.4°C most closely aligned with the survey results and was used to generate isolines from the complete weather data set using ArcGIS 9.1 (ESRI, Redlands, CA).

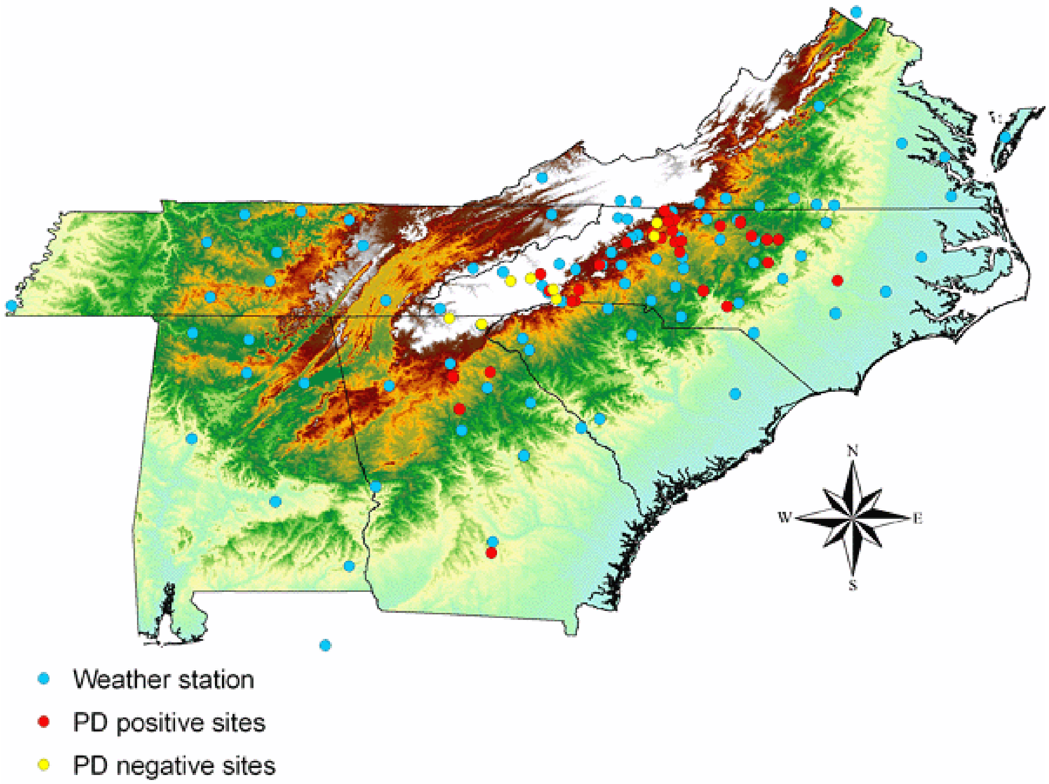


Fig. 3. Relief map of southeastern United States showing locations of weather stations and positive and negative Pierce's disease sites listed in Table 1 and their time of sampling.

Survey Results

Positive ELISA test results were obtained in 31 of the 38 vineyards tested (82%) in North Carolina and 6 of the 8 vineyards tested in Georgia (75%). Most of the vineyards testing positive were located at 400 m or lower elevation while most negative ones were near 600 m or higher. The incidence in most vineyards testing positive in 2001 or 2002 was only a few percent but in some vineyards over 30% of the positive vines were asymptomatic.

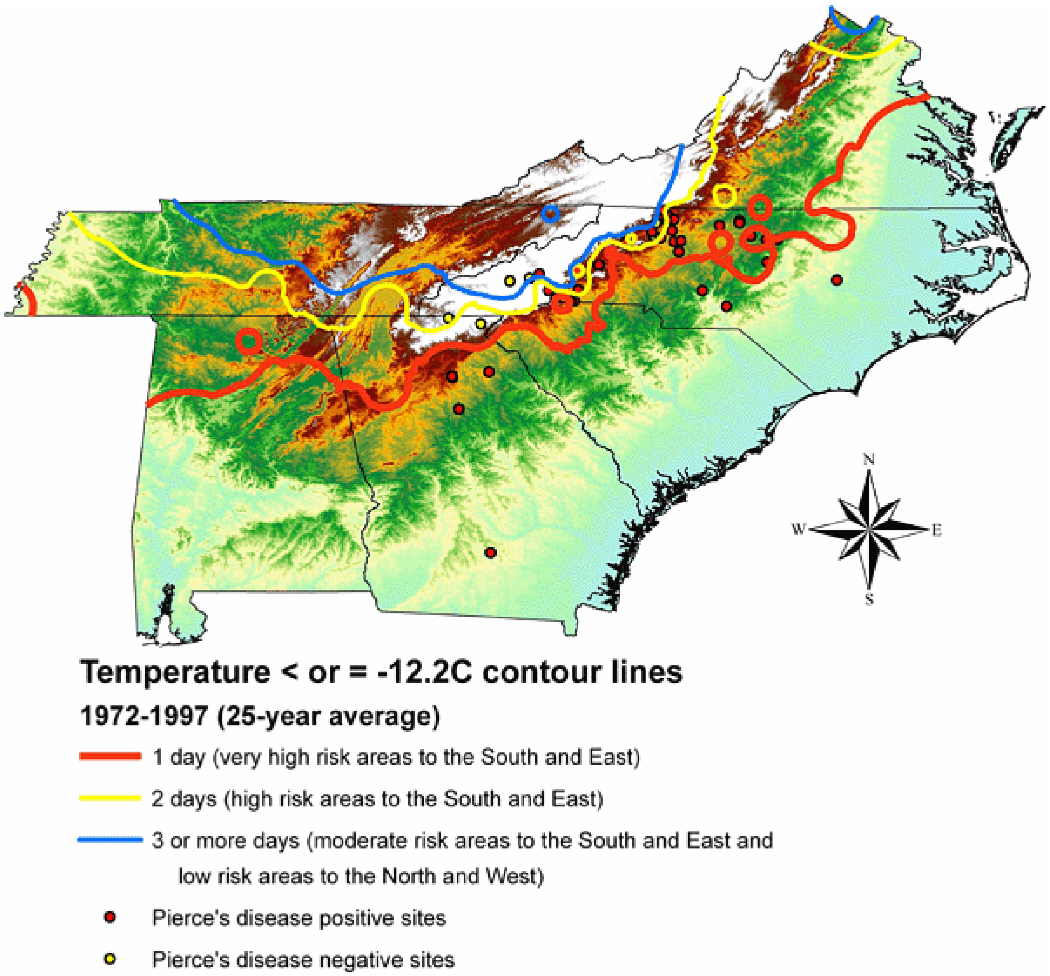
Although we may have overestimated the number of infected vines by considering those scoring 1 or 2 in our rating scale as infected, the detailed survey data still clearly document the increase in disease in the vineyards surveyed over time (Table 2). For example in 2001 in Hannover Park, the percent of vines scoring 2 to 4 ranged from 25.5% on Cabernet Franc, 14.3% on Cabernet Sauvignon, and 42.5% on Chardonnay. A large percentage of these symptomatic vines died in the winter of 2001-2002 or during the summer of 2002 and by September 2002, 17.3, 27.5, and 2.3% of the Cabernet Franc, Cabernet Sauvignon, and Chardonnay vines that scored ≥ 2 had died.

Catastrophic losses occurred in some vineyards. At Silk Hope the number of Chambourcin vines that died from PD increased from 6.9% in 2001 to 69.2% in 2003 (Table 2). At Rockhouse the percent of the Cabernet Franc that were dead increased from 0.7% in 2001 to 48.1% in 2006 to 53.5% in 2007 (Table 2). The detailed survey data indicate that in none of the years of the survey was there enough cold weather to "cure" infected vines (Table 2).

Relationship of Isotherms to PD Occurrence

Isolines for each of the temperature thresholds were determined for the years 1972-1997 (25-year winter average) (Fig. 4A and B), 1997-2005 (8-year winter average) (Fig. 5A and B), and 2004-2005 winter average (Fig. 6A and B) using ArcGIS 9.1.

A


N
W
E
S
Temperature < or = -12.2C contour lines
1972-1997 (25-year average)
1 day (very high risk areas to the South and East)
2 days (high risk areas to the South and East)
3 or more days (moderate risk areas to the South and East and low risk areas to the North and West)
Pierce's disease positive sites
Pierce's disease negative sites

B

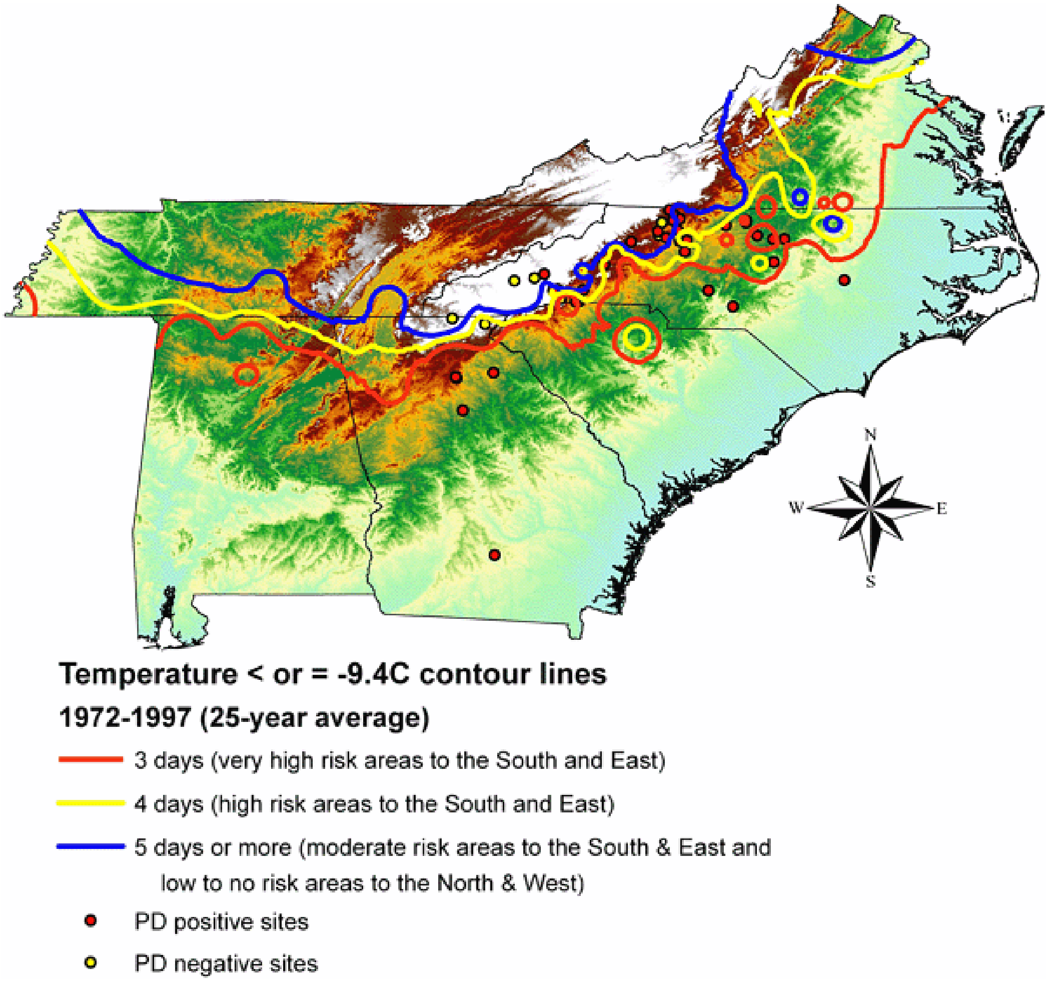
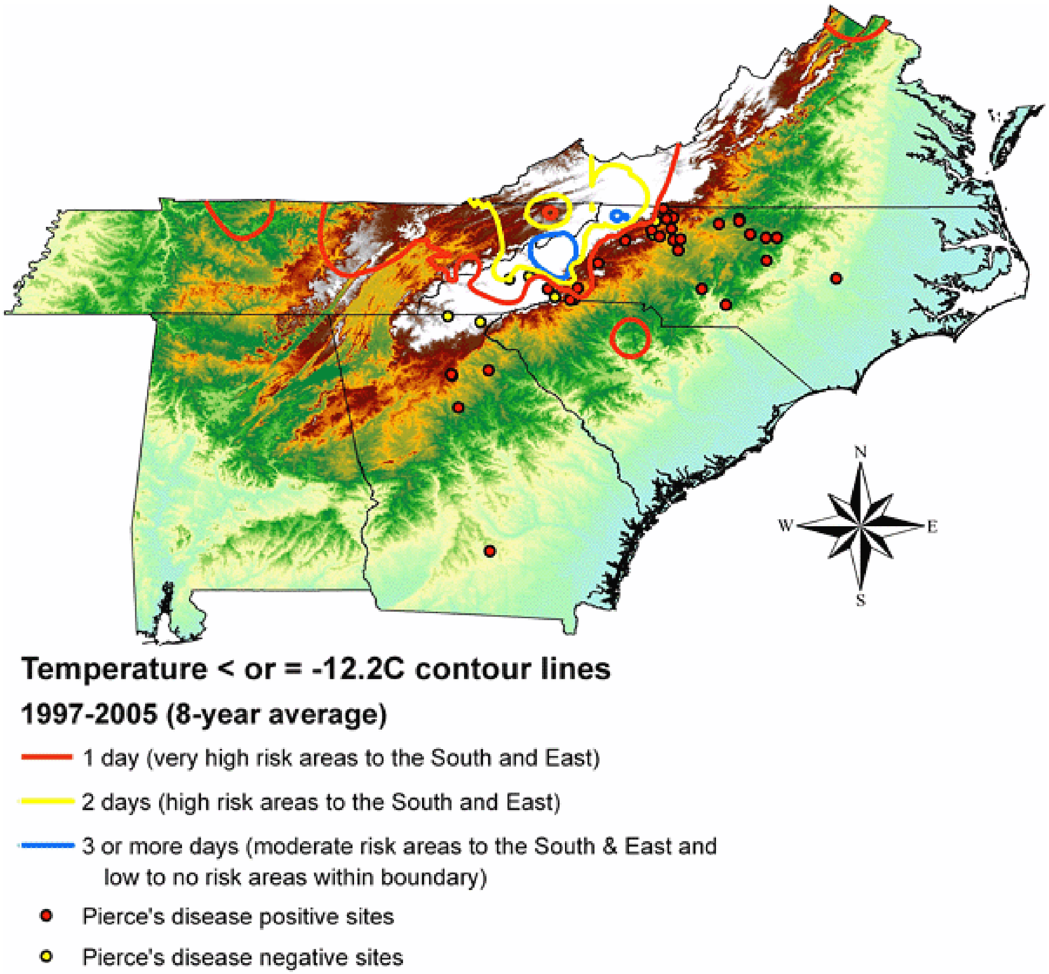


Fig. 4. Contour lines for Pierce's disease risk zones based on number of days of minimum temperatures 1972-1997 (25-year average): (**A**) ≤: -12.2°C contour lines and (**B**) ≤ -9.4°C contour lines.

A


N
W
E
S
Temperature < or = -12.2C contour lines
1997-2005 (8-year average)
1 day (very high risk areas to the South and East)
2 days (high risk areas to the South and East)
3 or more days (moderate risk areas to the South & East and low to no risk areas within boundary)
Pierce's disease positive sites
Pierce's disease negative sites

B

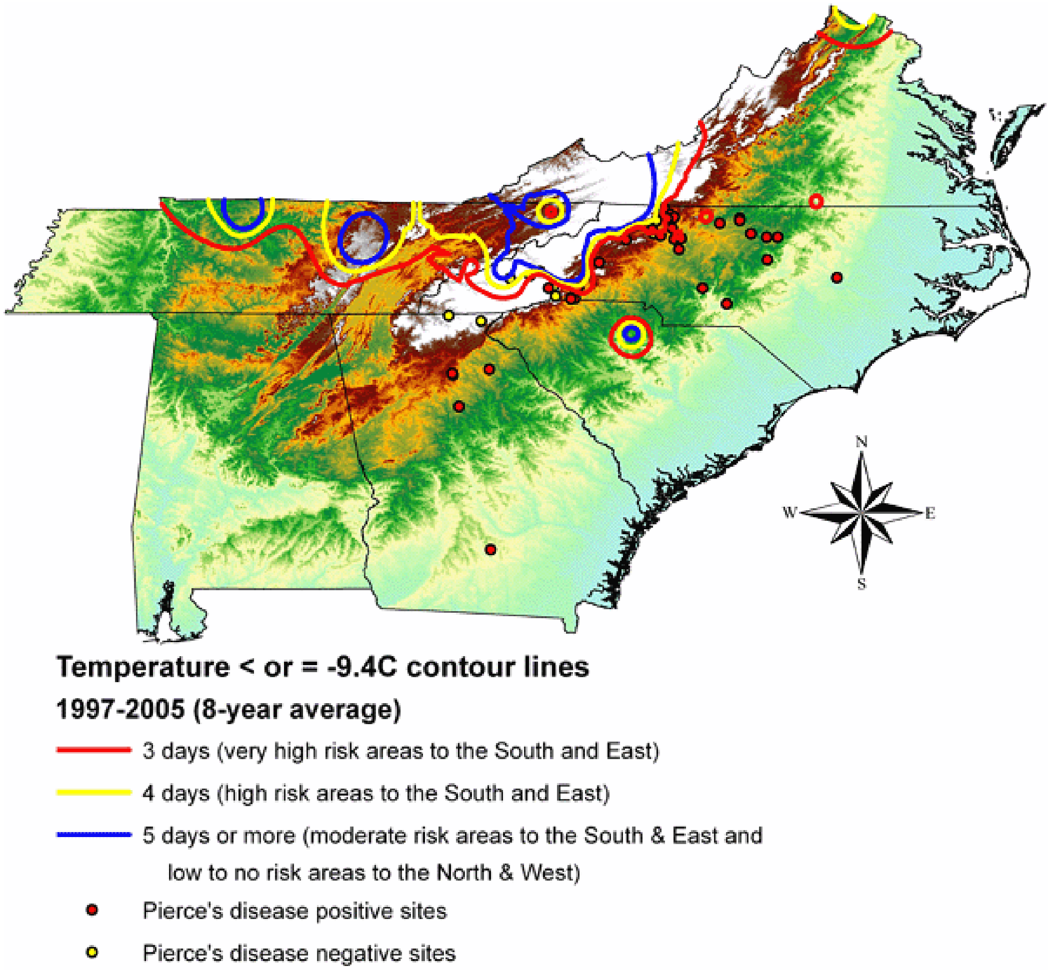
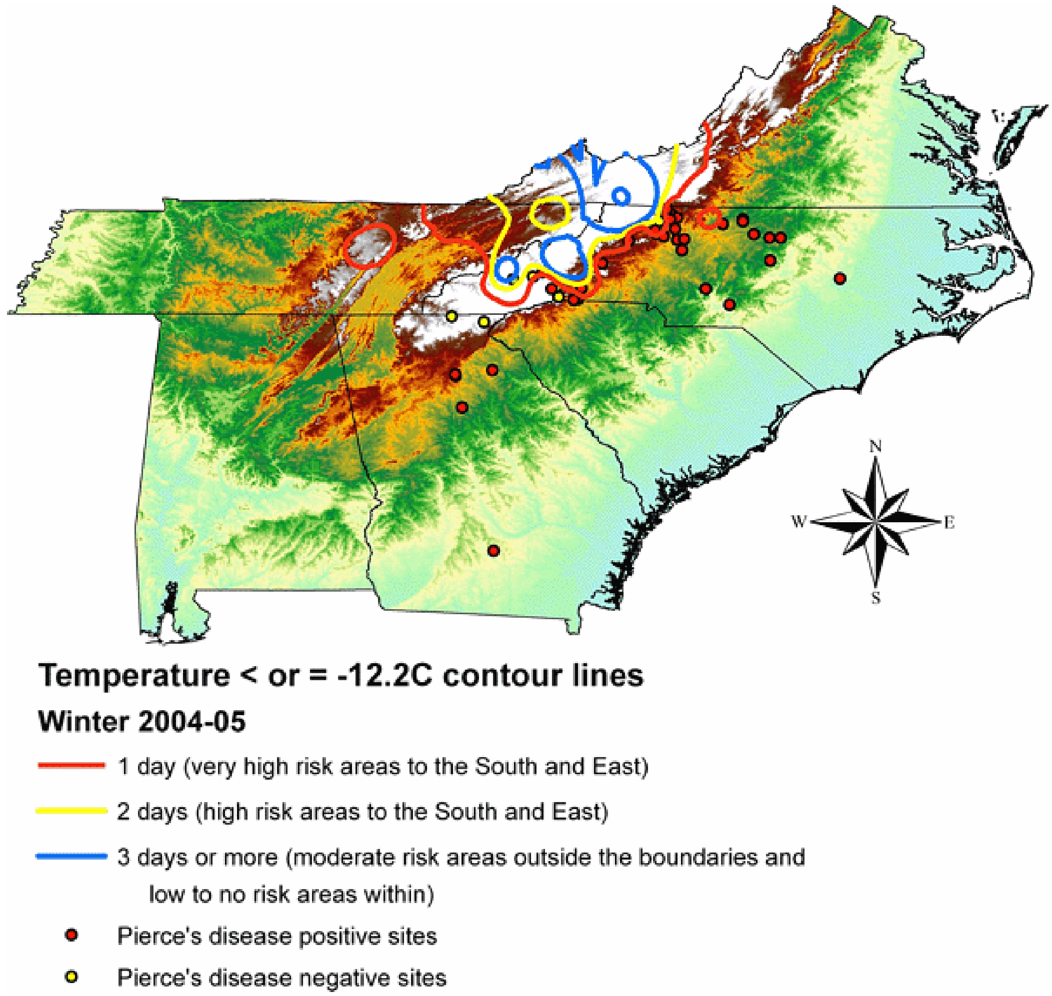


Fig. 5. Contour lines for Pierce's disease risk zones based on number of days of minimum temperatures 1997-2005 (8-year average): (**A**) ≤ -12.2°C contour lines and (**B**) ≤ -9.4°C contour lines.

A


N
W
E
S
Temperature < or = -12.2C contour lines
Winter 2004-05
1 day (very high risk areas to the South and East)
2 days (high risk areas to the South and East)
3 days or more (moderate risk areas outside the boundaries and low to no risk areas within)
Pierce's disease positive sites
Pierce's disease negative sites

B

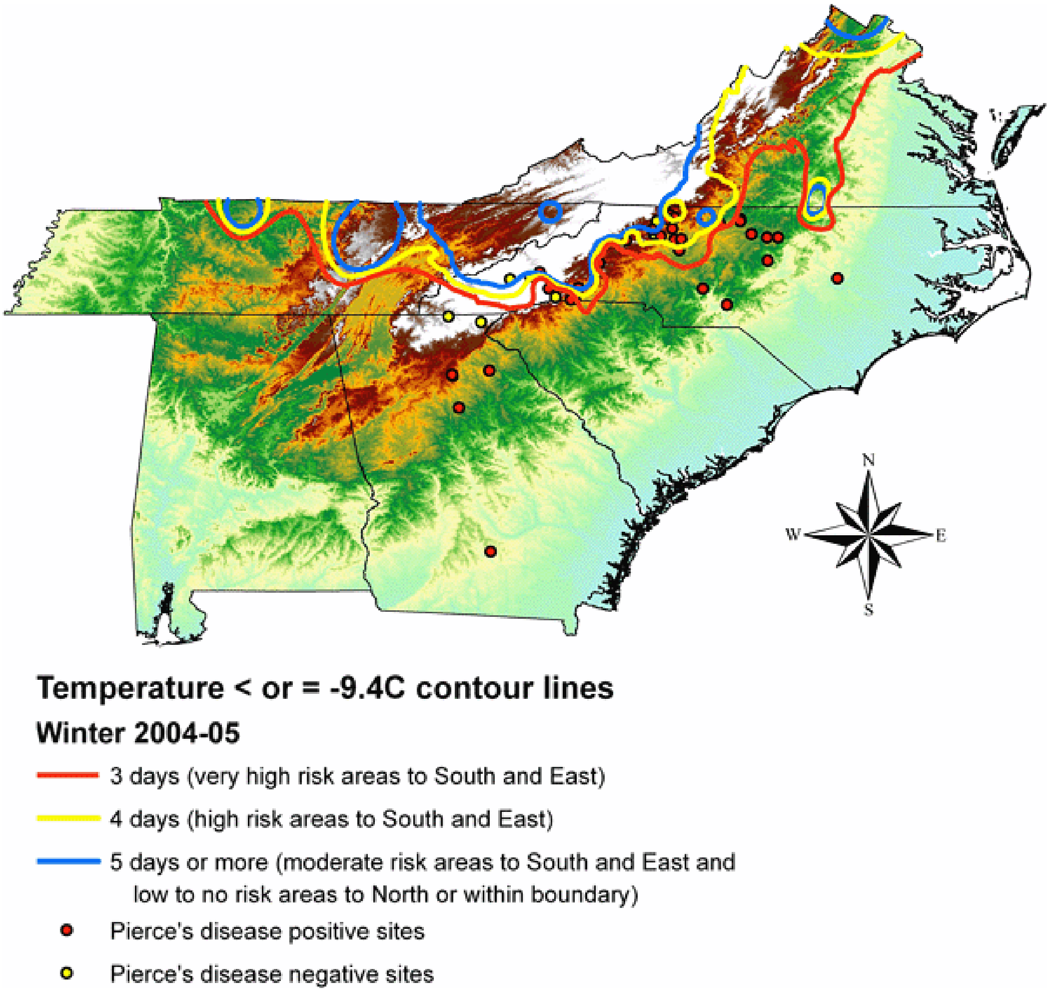


Fig. 6. Contour lines for Pierce's disease risk zones based on number of days of minimum temperatures 2004-2005 (average): (**A**) ≤ -12.2°C contour lines and (**B**) ≤ -9.4°C contour lines.

The regions of North Carolina and Georgia with vineyards in which no PD was observed or the PD incidence was very low most closely aligned with regions defined by the isolines that had >2 days of temperatures ≤ -12.2°C or >4 days of temperatures ≤ -9.4°C during the winter months for the period 1972-97 (Fig. 4A and B). Additionally most of Tennessee falls within the low risk area which supports observations that the disease is rare there (David Lockwood, *personal communication*). The high risk area, east and south of the red isotherm (Fig. 4A and B), most closely matches with the "occasional" but not the "severe" region in the 1980s map created by Purcell (Fig. 1), which is widely cited. However the disease is much more common in the Piedmont and southern Appalachian Mountains than indicated by "rare."

When isotherms for the period 1997-2005 were generated, the isotherms shifted significantly to the north and west (Fig. 5A and B) compared to the previous 25 years. This shift has been noticed by others; the United States Department of Agriculture has issued a new hardiness zones map which also reflects a warming trend from 1990 to 2006 (1). During the winter season of November 2004 to March 2005, we observed an even greater shift (Fig. 6A and B). These isotherms indicate that most of the winegrape production area in the Southeast is at risk if warm winter temperatures continue. The temperatures during the 2004-2005 winter season extend the PD risk area into most of Virginia. Recently, Wallingford and coworkers have found PD throughout much of the winegrape growing region of Virginia (18).

Conclusion

There is no shortage of documentation that the global average ground surface temperature has risen in the 20th century and a similar trend has been observed in the southeastern United States (Alabama, Georgia, North Carolina, South Carolina, Tennessee, and Virginia). Several reviews have been written recently on the potential impact of global warming on plant diseases and their management (3,8). However these reviews and most studies have focused on the long-term effects of climate change on plant pathogens. In this study we found evidence that PD of grapevines is increasing in severity in the southeastern US as a result of warmer winter temperatures over the past 6 years.

Our results indicate that PD is now a threat thoughout most of the winegrape growing region of the Southeast. The location of the isotherms also indicates that the threat may extend northerly well into states contiguous to those studied. Conversations with owners of vineyards established in the early and mid-1980s in North Carolina confirm that PD was rarely observed until the past 10 years. The survey data from the Rockhouse vineyard in the Cabernet Franc block also support this observation. The vineyard was first planted in 1996 and when we first surveyed it in 2001 only 0.7% of the vines were dead. However by 2007 53.5% of the vines were dead from PD (Fig. 7). A similar situation has been observed in Georgia. Vineyards planted above 300 m elevation typically lost < 20 vines per year to PD but over the past 2 years losses have been 400 to 500 vines per year (P. Brannen, *unpublished data*). This indicates that winter minimum temperatures in the past 6 years have not been cold enough to cure infected vines (6). Easterling et al. 1997 & 2005 and Karl et al. 2000 reported that the increase in the global temperature for most parts of the world over the past few decades appear to be manifested more strongly in daily minimum temperatures which are the night time lows rather than the daily maximum temperatures or the day time highs (4,5,11). If this is the case in the southeastern US, then it may help to explain the increase in PD severity we observed.

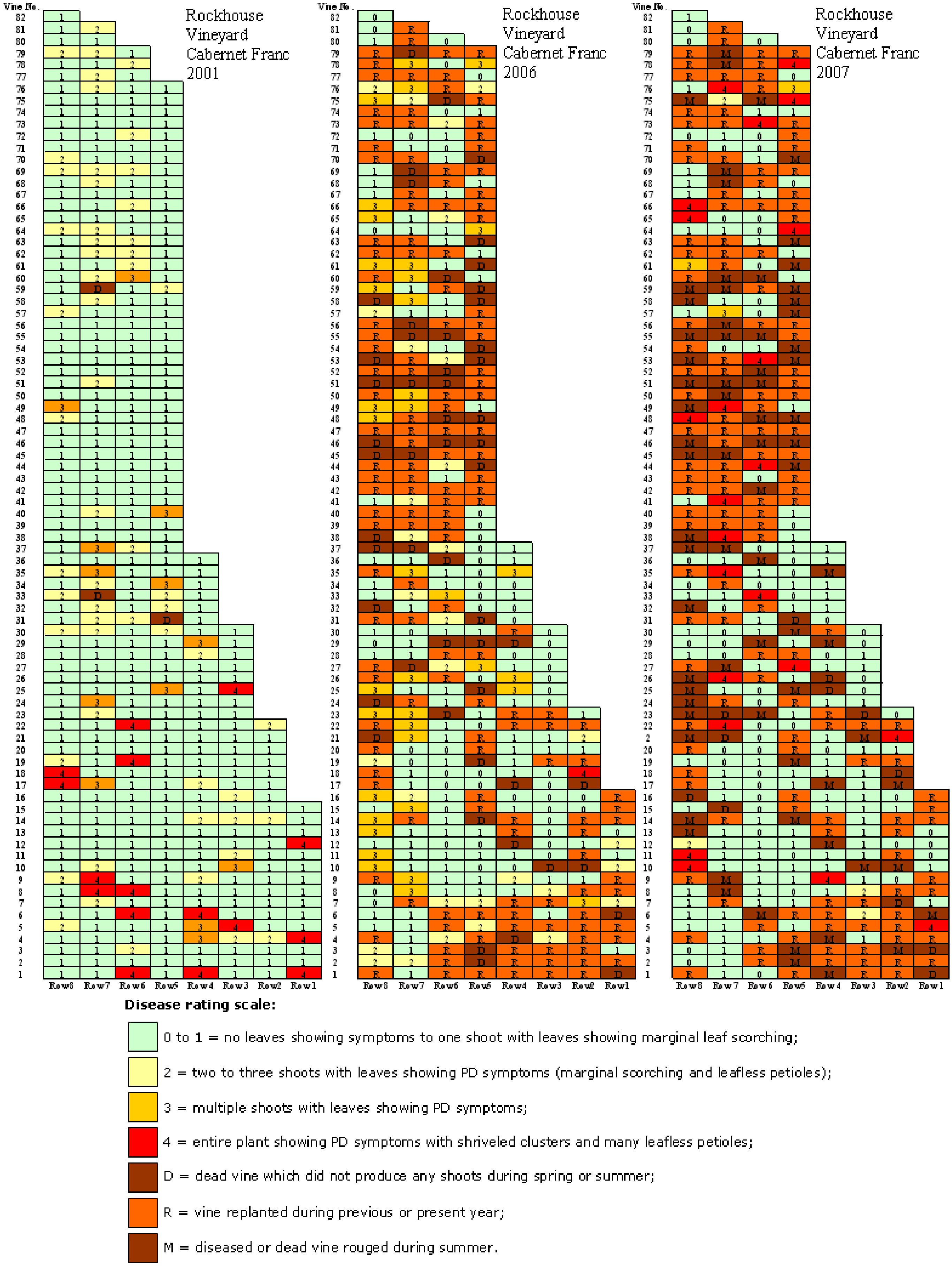


Fig. 7. Progression of Pierce's disease in a block of Cabernet Franc at Rockhouse Vineyard, NC in 2001, 2006, and 2007.

Various measures of temperature have been used to define areas at highest risk for PD. Alexander Purcell (Fig. 1) used average January minimum temperatures of 4.5, 1.7, and -1.1°C to define severe, occasional and rare zones. In Texas, < 800 chilling hours (hours of chilling at or below 7.22°C beginning with the first freeze) has been used to define the high risk areas (Jim Kamas, *personal communication*). In our study we found that days during the period November to March with minimum temperatures of ≤ -9.4°C or ≤ -12.2°C best defined the risk of PD. Both measures defined similar areas at high risk. Days with minimum temperatures of ≤ -12.2°C is more conservative and defines the zero (minimal PD) risk zone further north and west than days with minimum temperatures of ≤ -9.4°C.

Our maps should be used as a guide for those considering establishing vineyards in the southeastern US. They reinforce the threat of PD in the Coastal Plain and eastern/southern Piedmont of the states. However they also indicate that if the trend to warmer winters continues, there is an increasing threat to vineyards in the central and western Piedmont regions throughout the Southeast. Also as inoculum of *Xf* builds up in reservoir hosts, PD is likely to become more of a problem in states such as Virginia and Tennessee where it has been uncommon.

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