Evaluation of Chinese Chestnut Cultivars for Resistance to *Cryphonectria parasitica*

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ABSTRACT

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Seedlings of 13 Chinese chestnut cultivars were inoculated with and evaluated for resistance to *Cryphonectria parasitica.* Canker widths differed among the cultivars. Cankers increased for 1 week after inoculation but gradually constricted through callus formation; cankers on American chestnut controls expanded at a rate of 0.6 ± 0.3 mm per day until stems were girdled. Isolates of *C. parasitica* caused differential responses in Chinese and American chestnut.

Additional keywords: *Castanea dentata, C. mollissima,* chestnut blight

The Chinese chestnut (*Castanea mollissima* Blume) is an important species in world chestnut production and the best source of resistance to *Cryphonectria parasitica* (Murrill) Barr (4,8), the causal agent of chestnut blight. This fungus was introduced into North America at the beginning of this century on nursery stock of oriental chestnuts and spread throughout the natural range of the American chestnut (*Castanea dentata* (Marsh.) Borkh.). Within 50 years, blight destroyed several billion American chestnut trees (2). Efforts to transfer resistance from the Chinese to the American chestnut, primarily through an intensive backcross breeding program, are currently in progress (2).

The natural range of the Chinese chestnut extends from the far north of Jilin province (north 41°20′) to the tropical region of Hainan province (18°31′) in China. More than 300 cultivars are recognized and can be divided into five distinct regional groups based on horticultural traits and region of origin (10). A recent study on allozyme diversity among Chinese, American, and Seguin (*Castanea seguinii* Dode) chestnuts indicated that the Chinese chestnut is the most genetically diverse species (7).

Although *C. parasitica* is endemic to all major chestnut-growing areas of China (10), considerable variation in blight resistance has been reported (3,5,11). Zhao (11) surveyed 24 southern cultivars in the Nanjing area of China and found blight incidence to range from 0 to 63%. Most infected trees remained productive despite infection with *C. parasitica,* and yields were reduced more by poor orchard management, nutrient deficiency, and other disease and insect problems (10,11). Artificial inoculations and field observations of 12 *Castanea* species over a 30-year period showed the Chinese chestnut to be the most resistant (4). Given the apparent diversity of resistance within *Castanea mollissima,* it is important to use the best source of resistance in a breeding program. This study was undertaken to evaluate a diverse germ plasm collection of the Chinese chestnut for resistance to *C. parasitica.*

MATERIALS AND METHODS

The China National Chestnut Germplasm Plantation and Hubei Academy of Agricultural Science provided seeds of 13 traditional *Castanea mollissima* cultivars. Seeds were collected from isolated cultivar blocks after bulk pollination with pollen from five to 10 trees. Cultivars from four regional cultivar groups were included. Five cultivars (YeLiCang, JiuJiaZhong, Tiekeli, YangMaoLi, and DaHongPao) were from the Changjiang River region, four (WanBoKe, DuanZhi, YuCiMa, and LaoKuiLi) from the Southeast region, three (HongGuang, YangHong, and JianDingYouLi) from the Northern region, and one (ZhongGuohongPi) from the Southwest regional group. Seeds from four American chestnut trees near West Salem, Wisconsin (four half-sib collections) were included for comparisons.

All plants used in this study were 1-year-old seedlings. Seed were planted in 11.3-liter pots in a 6:1 (vol/vol) mixture of pine bark and sand containing 8.4 kg of Osmocote 17-7-12, 3.5 kg of dolomitic limestone, and 1.2 kg of Micromax per m^3. After a 2-month period in the greenhouse, seedlings were grown in full sunlight, sprinkler irrigated twice daily, and fertilized with Osmocote 17-7-12 as needed.

Three virulent *C. parasitica* isolates were used as inocula. Isolates SLA-155 and SLA-389 were provided by the Connecticut Agricultural Experiment Station. A third isolate (AL-W) was obtained from a Chinese chestnut on the Auburn University campus. Cultures were maintained on potato-dextrose agar (Difco) and were actively growing when used as inoculum.

Each seedling was wound-inoculated where the main stem diameter was at least

Table 1. Mean canker width of 1-year-old Chinese chestnut cultivar seedlings (*Castanea mollissima*) measured weekly after inoculation with three virulent isolates of *Cryphonectria parasitica* in 1992

Cultivar	Region	Mean canker width after inoculation (mm)					
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
YeLiCang	C.R.[a]	7.2[b]	6.8	5.1	3.8	1.9	1.5
HongGuang	N.	7.0	6.6	6.3	4.4	2.2	1.1
WanBoKe	S.E.	7.3	8.0	7.3	5.4	2.8	1.8
YangHong	N.	8.2	8.8	9.2	8.3	4.0	2.8
DuanZhi	S.E.	7.0	7.7	6.7	4.1	3.1	2.2
JiuJiaZhong	C.R.	8.0	8.6	9.2	7.1	4.1	3.5
TieKeLi	C.R.	8.1	9.3	8.2	5.7	3.9	3.5
YangMaoLi	C.R.	7.8	8.7	8.8	5.9	3.3	2.3
ZhongGuohongPi	S.W.	7.6	8.8	9.2	7.5	4.9	3.7
YuCiMa	S.E.	8.4	9.2	8.7	8.4	5.3	4.7
JianDingYouLi	N.	9.0	9.8	10.2	9.2	6.6	4.7
LaoKuiLi	S.E.	8.4	9.4	9.1	8.4	6.5	6.7
DaHongPao	C.R.	7.6	8.3	8.0	7.3	6.8	7.4
LSD							2.6

[a] C.R. = Changjiang River; N., S.E., and S.W. are north, southeast, and southwest regions of China.
[b] Each mean represents nine observations.

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10.5 mm. A sterile cork borer (3 mm diameter) was used to excise a disk of tissue exterior to the cambium. A disk of mycelium cut with the same instrument was placed in the stem wound, which was immediately sealed with Parafilm to prevent desiccation. Disease development was assessed as the vertical and horizontal expansion of visibly infected stem.

Nine seedlings from each of the 13 *Castanea mollissima* cultivars and each of the four *Castanea dentata* half-sib collections were randomly divided into three groups of three seedlings each. Each group was inoculated with one of the three *C. parasitica* isolates on 30 August 1992. The length and width of visible canker necrosis was measured every week for 6 weeks (30 August to 21 October). Healthy callus was not included in the canker dimensions.

Four *Castanea mollissima* cultivars and the four *Castanea dentata* half-sib collections were reevaluated in 1993. Five healthy 1-year-old seedlings per cultivar or collection were inoculated with one of the three isolates on 24 May 1993. A total of 15 seedlings per cultivar or collection were inoculated. A completely randomized design was used each year, and canker dimensions at the last recording date were subjected to an analysis of variance (9).

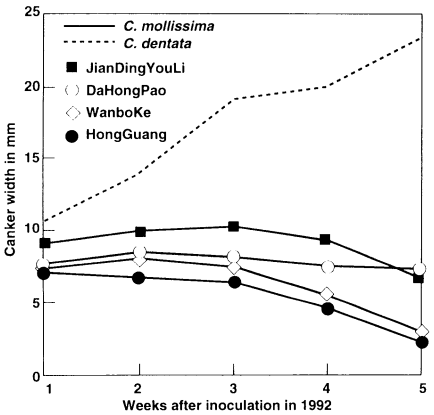

Fig. 1. Mean canker width of 1-year-old seedlings of selected Chinese chestnut cultivars and American chestnut collections after inoculation with one of three isolates of *Cryphonectria parasitica* in 1992.

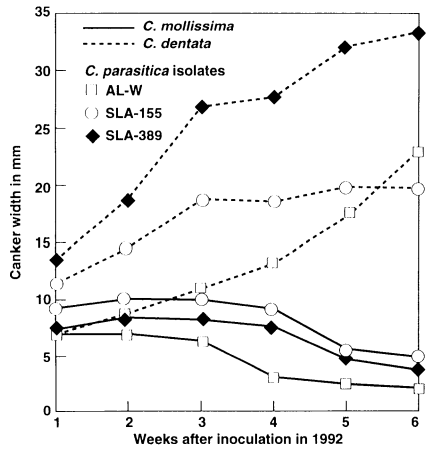

Fig. 2. Effect of *Cryphonectria parasitica* isolate on mean canker width of 1-year-old seedlings of four Chinese chestnut cultivars and four American chestnut collections in 1992.

RESULTS

Canker width and length differed among the Chinese chestnut cultivars inoculated in 1992 with *C. parasitica*. Since canker widths and lengths were correlated (r = 0.85, $P < 0.001$, 5 weeks after inoculation), only canker width will be presented. On most cultivars, canker width increased rapidly the first week after inoculation, after which it was reduced by callus formation (Table 1, Fig. 1). Many cankers became constricted or gradually walled off by callus tissues in the third week after inoculation. Callus necrosis was seldom observed. Six weeks after inoculation, average canker width among Chinese chestnuts varied from <2 mm on YeLiCang, HongGuang, and Wanboke to 2 to 4.7 mm on YangHong, DuanZhi, JiuJiaZhong, Tiekeli, YangMaoLi, ZhongGuohongPi, and YuCiMa and 6.7 to 7.4 mm on LaoKuiLi and DaHongPao. The stems at the inoculation wound on individual seedlings of YeLiCang, HongGuang, and Wanboke were completely healed 4 weeks after inoculation.

Canker growth did not differ among the half-sib collections of American chestnut. Cankers expanded linearly (Fig. 1) with an average expansion rate of 0.6 ± 0.3 mm/day from 30 August to 21 October 1992. Little or no callus formation was detectable. Most seedlings (70%) were girdled within 3 weeks and died.

Canker width differed for isolate in both species in 1992 (Fig. 2). Cankers were significantly larger in the American seedlings inoculated with isolate SLA-389 (average expansion rate of 1.3 mm/day between 30 August and 21 September) than with SLA-155 (rate of 0.9 mm/day) and AL-W. Canker widths in the Chinese chestnut seedlings inoculated with SLA-389 and SLA-155 were similar (Fig. 2). However, cultivar × isolate interactions were significant 5 and 6 weeks after inoculation.

Canker dimensions observed in both species in 1993 were similar to dimensions observed in 1992 (Figs. 3 and 4). Significant differences in canker widths were found among the Chinese cultivars (Table 2, Fig. 3), but not among the American collections. Similarly, a highly significant isolate effect on canker growth was detected in 1993 in both species (Fig. 4).

The combined analysis for canker widths in the four Chinese cultivars and four American collections over 2 years showed a nonsignificant effect of year and year × cultivar interaction 5 weeks after inoculation, but highly significant year × isolate and year × isolate × cultivar effects.

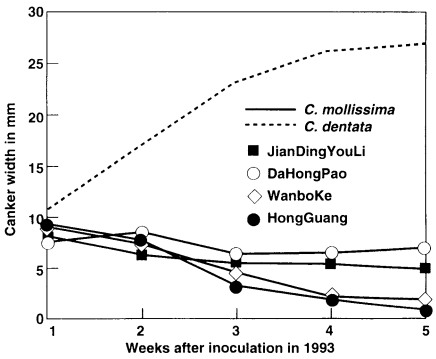

Fig. 3. Mean canker width of 1-year-old seedlings of selected Chinese chestnut cultivars and American chestnut collections after inoculation with one of three isolates of *Cryphonectria parasitica* in 1993.

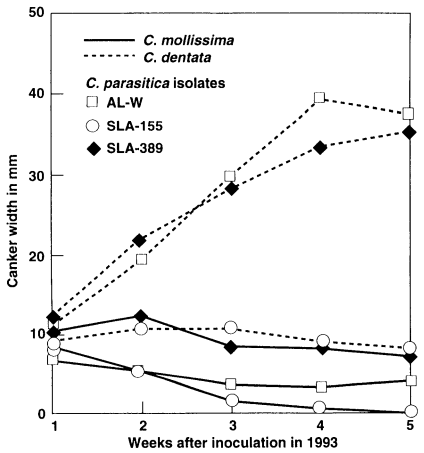

Fig. 4. Effect of *Cryphonectria parasitica* isolate on mean canker width of 1-year-old seedlings of four Chinese chestnut cultivars and four American chestnut collections in 1993.

DISCUSSION

In the present study, canker growth on the American chestnuts inoculated with

Table 2. Mean canker width of 1-year-old seedlings of four Chinese chestnut cultivars inoculated with three virulent isolates of *Cryphonectria parasitica* in 1993

		Mean canker width after inoculation (mm)				
Cultivars	**Region**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
HongGuang	North	8.4[a]	7.2	4.3	2.8	1.3
WanBoKe	South	8.3	7.4	4.5	3.3	2.2
JianDingYouLi	North	8.3	7.5	7.2	6.7	5.4
DaHongPao	Changjiang	7.6	8.4	6.9	6.6	6.7
LSD						3.1

[a] Each mean represents 15 observations.

isolates SLA-155 and SLA-389 was similar to that reported by Anagnostakis (1) (1.0 mm/day for SLA-155 and 0.9 mm/day for SLA-389 between 2 July and 19 September 1992) and Hebard et al. (6). This indicates that isolates were virulent and that American chestnuts were susceptible.

A range of resistance was detected among the 13 Chinese chestnut cultivars, confirming results of Headland et al. (5) and Zhao (11). The most resistant cultivars were HongGuang from the northern region of China and WanBoKe from the southeastern region, which should be considered as sources for future breeding programs. Although only a small percentage of the recognized Chinese cultivars were evaluated, different degrees of resistance occurred within and between regions. Our results should serve as benchmarks for future organized evaluations of blight resistance.

Isolate effects ($P < 0.001$, 1992 and 1993) were detected both years in Chinese and American chestnuts. In general, inoculation with SLA-389 resulted in larger cankers (mean canker width of Chinese and American seedlings 5 weeks after inoculation of 20 mm) than inoculation with AL-W (17 mm) or SLA-155 (7 mm). The significant isolate × cultivar interactions ($P < 0.01$ in 1992, $P < 0.001$ in 1993) suggest that canker expansion is related to specific combinations of resistance and virulence.

Even though similar canker widths were found both years, the significant year × isolate ($P < 0.001$) and year × cultivar × isolate interactions ($P < 0.001$) indicate that environmental factors affect host–pathogen interaction. The genetic component of blight resistance is poorly understood (8), but an examination of the full range of resistance within *Castanea* should determine the best sources for incorporation into *Castanea dentata.*

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