Summary of NE-140 Meeting, Natural Bridge State Park, Slade, KY (1985)

Gary Griffin:VPI

-He has found dsRNA in his continuous clearcuts in the Jefferson National Forest. Hardwoods will be cut again this winter in one of two plots to permit chestnut to grow under minimum competition. Two similar research areas are maintained in the Monongahela National Forest, Parsons, WV. Only a few superficial cankers have been found to date.

-An American Phytopathological Society monograph has been written entitled "Chestnut Blight, Other Endothia Diseases and the Genus Endothia. Galleys of the monograph are currently being made in St. Paul and should be done by December, 1985.

-No discussion of the contorversy of Endothia vs. Cryphonectria.

Dennis Fulbright:Michigan State

-They have isolated dsRNA-containing Endothia parasitica strains from over 20 American chestnut groves in Michigan (and Canada, too). Of the chestnut groves monitored each year, parts of the Grand Haven grove are looking worse than three years ago, but other parts of the grove are looking better or are the same. The County Line site (visited by the Ne-140 group in 1982) is looking better than ever. The Roscommon site (northern mid-Michigan) is much better than 1980.

-CL25, an isolate recovered from Crystal Lake is hypovirulent. The nature of this Hv is cytoplasmic and transmissible with no obvious abnormal culture morphology. The Hv determinant(s) is maternally inherited through the ascospore. No dsRNA has yet been detected using CF11 columns, however, a very faint band has been observed in gels following a technique used for DNA plasmid isolation.

Cankers initiated with a Virulent isolated and treated with CL25

| Virulent | Treated with C25 | No treatement with CL25 |
|---|---|---|
| CL 1 | 1210 mm | 5650 mm |
| CL 2 | 2760 | 3120 |
| CL 3 | 6300 | 5480 |

-Superinfection studies, where all dsRNA's from the Michigan standard strains GH 2, GHU 4, and RC 1 into one strain, have not worked out. Three distinct banding patterns have not been observed and new patterns continue to emerge.

-(Cindy Paul) Hybridization studies continued using another Michigan dsRNA as a probe to dsRNA from Michigan, other North American (Tenn, WV) and European strains. The dsRNA from this isolate, RC 1 showed little homology to ds RNA from any strain but itself. When less stringent conditions were used to allow dsRNAs that may be somewhat unlike to hybridize: with GH 2 as a probe, it barely began to show bands with the other Michigan isolates-they overexposed the X-ray film and got so much background that the autoradiograms were hard to read. She thought it was possible within a localized area to use a quick dot blot for homology.

GH 2 dsRNA did hybridize with dsRNA from the County Line site

GH 2 " did " " " with another GH isolate with a different banding pattern

GH 2 dsRNA did not hydridize with dsRNA from France, Italy, or Tennessee

GH 2 dsRNA did not " " 9-B-2-1 or BF 5 from WV
GH 2 dsRNA did hybridize with dsRNA from Frankfort and Rockford
RC 1 dsRNA only hybridized with itself

-(Frank Ewers-tree physiologist) He took hydraulic conductivity (hc) measurements and dye ascents from V and HV infected trees.

| Strain | Time 0 | One month | Two months |
|---|---|---|---|
| CL 116 V | 12 | 1 | 0 |
| CL 25 Hv | No effect on hc ---------- | | |

Said Ghabrial:University of Kentucky
-The weight for each major band in Ep 713 is 4.5-6 million daltons-the lower band is 750 bp.
-The lower band of Ep 713 lights up all 5 bands from Ep 713 and nothing from the total nucleic acids from the genetic parent, Ep 155.
-He thinks that some dsRNA from certain systems-some of the bands may be deletion mutations from larger dsRNAs, because they have the same termini.

Shiv Hiremath:UK
-He cloned a fragment of dsRNA from Ep 713 (French) which probably represents a terminal sequence
-This cloned fragment hybridizes to all Ep 713 bands, weakly to Ep 780 (Italian) and not at all to Ep 905 (American Hv) and Ep 155 (American V).

Lou Shain:UK
-He initiated cankers with Ep 289 (a virulent isolate-methionine requi and converted them at 12 weeks with a debilitated Hv strain, and then examined th movement of the Hv. He photographed the cankers at time of Hv inoculation. At 3, 6, and 9 weeks after Hv inoculation, he induced cirrhi and then sampled both the cirrhi and the underlying bark and cultured them on PDA. He got all virulent isolates from the spores and mostly Hv isolates from the underlying bark.

Jack Elliston:CAES
-He talked about his sampling in Michigan in 1982-the samples he collected ar still frozen in New Haven. He sampled 3 areas in Michigan, looking for both Endothia parasitica and other fungi. He gets some very, very slow growing E.p. isolates from Kalkaska County and mostly Penicillium and Trichoderma sp.
-He discussed the 72 three-year old American chestnut stems at the Lockwood Station Farm, CT. They treated all cankers with a slurry of 10 isolates every fo months for 4 years-up until 1981, when the virulent cankers got out of hand. Fifty percent of the trees are still alive as of 1985. A duplicate plot was set up outside New Haven (72 trees treated with the slurry) and sampling there produc mostly virulent E.p. Of the non-virulent isolates, by far the most common isolat was E.p. 60-like, which he found surprising since E.p. 60 is so unlikly to spread

Neal VanAlfen:Utah State
-He is still looking for polymerase, because he felt a polymerase would lend support that is a virus involved.
-He is trying to find out what the dsRNA is doing to E.p., so he continuously transfers Hv isolates until he eliminates the dsRNA-he now has white mutants that mimic Hv isolates but do not contain dsRNA
-He wonders whether certain mRNAs are being shut off by the dsRNA.
-He has had little success with cell-free infection. He has tried liposomes and vesicle fusion with protoplasts. He gets dsRNA into the protoplasts but he fi

it hard to maintain them, so he is trying to set up for microinjection to try to inject dsRNA into hyphae.

Sandy Anagnostakis:CAES

-She mapped and is following 75 trees on a 2 acre tract. She sampled all cankers at various time intervals, converted the virulent isolates in the lab wi a slow growing Hv isolate and then took the convert back to the tree to convert the virulent thallus. She did this on a one-on-one basis for a while, until it became too much work

| | Cumulative cankers in the plot | | | |
|---|---|---|---|---|
| | 1982 | 1983 | 1984 | 1985 |
| Number of cankers | 12 | 57 | 161 | 256+ |
| Number of v-c groups | 6 | 11 | 38 | 42+ |

Beginning this year she began spraying with a Hv conidia suspension (based on George Kuhlman's results). She took three of the isolates and converted them with Italian Hv and will monitor her results.

. -She began spore trapping in April 1985, at three week intervals, and found no E.p. until September. She has found a number of slugs and has been able to culture E.p. from them.

Bill Carey:Duke

-He looked at 22 chestnut trees 20 cm or greater (dbh). His sampling scheme was set up in a cross fashion, with a 30 m circle around each of the 22 test tree

He sampled all E.p. he found on chestnut and all other hosts, within the 30 m cir and in the area of a cross-152 m long (5 m wide) in each cardinal direction from He found that 57% of all scarlet oaks were infected. Of all the E.p. isolates th he cultured, only 2 looked abnormal. (He did not examine for dsRNA).

Colin McKeen:Canada

-He has been looking around southern Ontario for E.p. and found three locatio with abnormal isolates and Dennis Fulbright examined some of these isolates for dsRNA, and found it in several of the isolates. He did a pathogenicity test in golden delicious apple and found the suspect isolates produced a much smaller les

William MacDonald:WVU

-He reported on the one year cankers from Dave Hobbin's study. Rick Sypolt, Assistant Professor from Glenville State College spent the month of July, 1985 single sporing from those cankers. He classified isolates as either white (brown italian) or brown (similar to 5-9-1B). He found that white isolates were more frequent in the hypovirulent-compatible treatments than in the hypovirulent-incompatible treatments. The whites also appear more frequently in the outer portions of the canker than in the inner portions of the same canker.

Number of Brown and White Isolates at Various Levels for the HC Treatments

| | Inside (position 1-8) | | Outside (position 9-12) | |
|---|---|---|---|---|
| Level | Number of White Isolates | Number of Brown Isolates | Number of White Isolates | Number of Brown Isolates |
| 0.5m | 2 | 58 | 1 | 58 |

| | | | | |
|---|---|---|---|---|
| 1.0m | 9 | 79 | 37 | 53 |
| 1.5m | 4 | 86 | 54 | 35 |
| 2.0m | 0 | 90 | 56 | 31 |

Number of Brown and White Isolates at Various Levels for the HI Treatments

| | Inside (position 1-8) | | Outside (position 9-12) | |
|---|---|---|---|---|
| Level | Number of White Isolates | Number of Brown Isolates | Number of White Isolates | Number of Brown Isolates |
| 0.5m | 3 | 117 | 5 | 115 |
| 1.0m | 2 | 117 | 38 | 72 |
| 1.5m | 0 | 120 | 5 | 115 |
| 2.0m | 0 | 120 | 0 | 120 |

The thought after these results were obtained that these were mass pycnidia and consequently it could not be determined whether both types of isolates (brown and white) were present in the same pycnidium. Rick picked four cankers to examine more closely, and from single pycnidia he found both brown and white isolates in the same pycnidia. He picked 30 single spores from each pycnidium and found whites and browns ranging from 26 white/4 brown to 3 white/27 brown. He did not find any pycnidia that were totally white or totally brown.

-He reported on chestnut blight in Minnesota, found in several stands in the southeastern portion of the state. The trees, when not affected by the blight do not look vigorous, possibly due to the harsh winters. Those stands which do have the blight have a severe case, and the Dept. of Natural Resources of Wisconsin is putting the heat on Minnesota to destroy the infected stands, and they are due we of some of Wisconsin's finest chestnut stands, and fear thes beautiful stands hav the chance of becoming infected from spores carried in the prevailing wind from Minnesota. Dave French from the University of Minnesota wants advice on what to do with these stands. A discussion followed MacDonalds presentation, and there was a sharp difference of opinion. Dennis Fulbright felt that erradicating the stands was not the direction to go, since we as plant pathologists looking at controlling chestnut blight with biological control agents are not in the busines of cutting and burning. Gary Griffin on the other hand had visited the stands in Wisconsin and said that they rivaled anything he has seen in Michigan and he felt that anything we could do to favor those stands in Wisconsin should be done-he suggested cutting the stems and pulling out the stumps and burning the entire tre MacDonald said he would relay the discussion to French for his consideration.

Double:WVU

-He reported on a pathogenicity study involving some of the State Survey isol that contain dsRNA. Some of the isolates were more pathogenic than 5-9-1B, but no significantly so.

| Isolate | Canker area (L+W/2) | # of 37 v-c groups converted |
|---|---|---|
| State Survey 6-1 | 15.47a | 1 |
| 39-a-1-1 | 13.74a | not tested |
| Atypical brown* | 12.95ab | not tested |
| 5-9-1Brown | 12.56ab | - |
| State Survey 13-8 | 10.26abc | 0 |
| State Survey 1-3 | 10.03bcd | 0 |
| Dody Dunning | 9.48cd | 2 |
| Brushy Fork 5 | 7.24d | 5 |

| | | |
|---|---|---|
| State Survey 15-19 | 3.06e | 6 |
| 9-b-2-1 | 1.32e | not tested |

*an atypical isolate from Dave Hobbin's study

-He reported on the media problem. Studies indicate that some isolates show marked differences in growth to different PDA lots, while other isolates differe with D,L versu L-Methionine, and some isolates looked different grown in Brooks versus the Agricultural Sciences building. Studies are continuing.

NE-140 Pathogenicity Study

-Jack Elliston's results

| | | | Canker area | Total Virulence Ranking (contains stromata) |
|---|---|---|---|---|
| SOSM | Duke | G | a | a |
| Ep 155 | CAES | D | a | ab |
| CR | VPI | F | bcd | bc |
| Ep 523 | CAES | H | bcd | bc |
| WK | VPI | A | bcd | cd |
| 5-9-1 | WVU | B | abc | cd |
| BLBO | MSU | E | cd | d |
| CL116 | MSU | C | bcd | e |

-West Virginia's results

| Strain | Canker area | Total virulence units |
|---|---|---|
| D | 376a | 838a |
| A | 267c | 704b |
| H | 286bc | 689b |
| B | 329ab | 658b |
| G | 271c | 593b |
| F | 142d | 294c |
| E | 162d | 256c |
| C | 159d | 208c |

Elliston agreed to write up the results for publication with statistics on: canker area, number of stromata, number of stromata with perithecia and total virulence units (Canker area+ # stromata/10 + # stromata with perithecia/10).

The NE-140 meeting in 1986 with be at Mt. Lake Park, Virginia.