Chemical Fingerprinting:

A POTENTIAL TOOL FOR IDENTIFYING DISEASE RESISTANT CHESTNUT TREES

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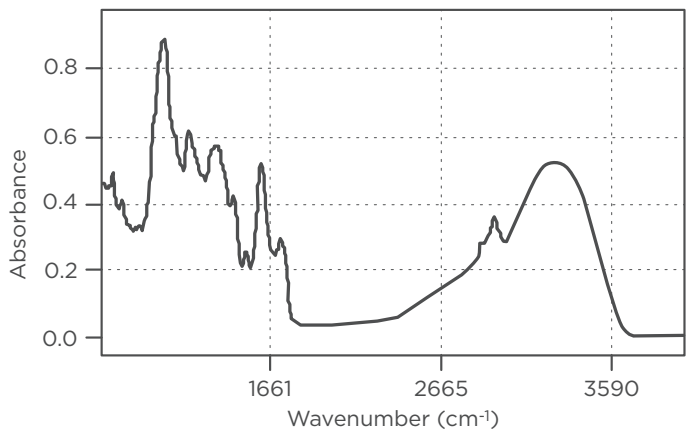
Resistant trees are one important strategy for managing diseases, such as chestnut blight and Phytophthora root rot (PRR). However, waiting for disease symptoms to develop in order to identify resistant trees can be a lengthy process. So, alternative, more rapid approaches for identifying disease resistant trees are needed.

One such approach is chemical fingerprinting. Chemical fingerprinting provides a snapshot of the chemical composition of a plant tissue or extract at a given time. Infrared (IR) spectroscopy is one method of chemical fingerprinting. IR spectroscopy measures changes in the absorption of IR light by different chemicals over specific windows of the electromagnetic spectrum (e.g. the mid-IR spectrum runs from 400 - 4000 cm^{-1} or 25000 - 2500 nm). When analyzing complex plant samples like extracts from chestnut trees, individual or specific chemicals present within a sample cannot be identified. What this approach can do is pick up on differences in the types of chemicals and their concentrations. By measuring how different chemical groups respond after being hit with IR light, a unique spectrum or chemical fingerprint for each sample is generated (**Figure 1**).

Chemical fingerprinting may be useful for identifying disease-resistant trees, because plant-produced chemicals are known to be important components of how plants defend themselves against pathogens. Moreover, genetics and environmental factors can impact the levels of chemicals present within a tree. The levels and types of chemicals can change over time, including in response to pathogen infection. So, chemical fingerprint data can be combined with disease phenotype data (e.g. whether a tree is resistant or susceptible) to develop models for predicting if a tree is likely to be resistant or susceptible based on its chemical fingerprint.

Figure 1

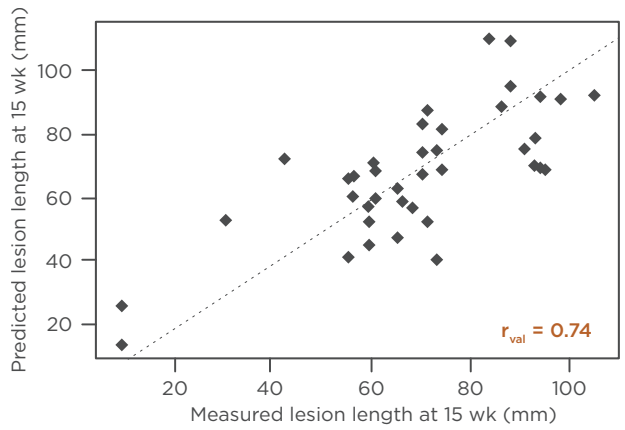


A representative chestnut chemical fingerprint from 700 – 4000 cm^{-1}.

Evaluating chemical fingerprinting as a tool to screen hybrid chestnut for disease resistance

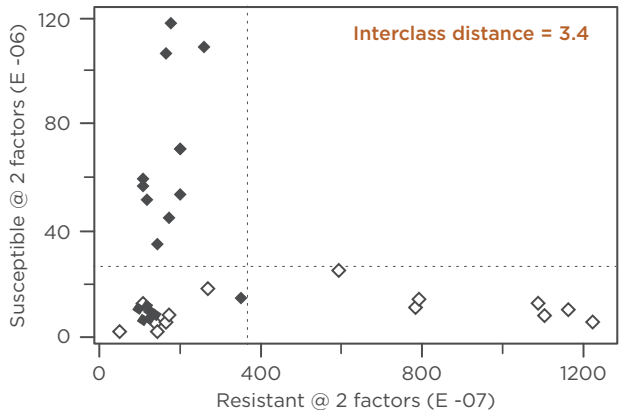
With funding from The American Chestnut Foundation (TACF), the use of chemical fingerprinting for screening hybrid chestnut for disease resistance and susceptibility was evaluated. Albert Abbott (University of Kentucky), C. Dana Nelson (U.S. Forest Service), Pierluigi (Enrico) Bonello (Ohio State University), and Luis Rodriguez-Saona (Ohio State University) were co-principal investigators on this project. To test this approach, non-infected stem tissue from BC_3F_2 and BC_3F_3 hybrid families which had been traditionally screened for resistance to blight and/or PRR were analyzed. Tissue and phenotypic data were provided by Jared Westbrook (TACF), Tetyana Zhebentyayeva (The Pennsylvania State University), and Stephen Jeffers (Clemson University).

Figure 2



Correlation plot from 7-factor PLSR model showing the relationship between predicted and measured blight lesion lengths for 'Clapper' BC_3F_3 hybrids (N = 40 excluding samples removed based on preliminary analysis). rval: correlation coefficient of cross validation. The closer rval is to one the stronger the correlation is between predicted and measured values.

Figure 3



Two factor SIMCA can distinguish between samples from PRR-resistant (closed) and susceptible (open) trees (N = 31 excluding samples removed based on preliminary analysis). The interclass distance between resistant and susceptible trees is 3.4 (the higher the value the less likely the model is to classify samples as both resistant and susceptible).

To collect chemical fingerprints, two undergraduate researchers at Ohio State University, Lauren Schnitkey and Caleb Mathias, finely ground stem tissue and extracted it with methanol. Then they concentrated extracts and analyzed them using a Fourier-transform infrared (FT-IR) spectrometer over the range of 700 – 4000 cm^{-1}. Chemometrics (statistical analysis of chemical data) was performed to evaluate whether chemical fingerprints can be used to predict hybrid chestnut susceptibility to blight or PRR. Two different statistical methods were tested: soft independent modeling of class analogy (SIMCA) and partial least squares regression (PLSR). SIMCA develops models for classifying samples into different groups (e.g. resistant or susceptible), while PLSR allows for the prediction of quantitative traits, like lesion length, another measure of susceptibility.

Initially, chemical fingerprints from different resistance sources – 'Clapper' and 'Graves' – were grouped together for statistical analysis. However, preliminary tests revealed that the accuracy of chemical fingerprint-based predictions improved when samples from different sources were analyzed separately. For 'Clapper' derived BC_3F_3 hybrids, two spectral regions from 1072 – 1618 and 744 – 1001 cm^{-1} were useful for predicting variation in the length of blight lesions. Moreover, there was a strong positive correlation between measured lesion lengths and predicted lesion lengths based on chemical fingerprint data (**Figure 2**). Whereas for 'Graves' BC_3F_2 hybrids, the region from 1001 – 1029 cm^{-1} was important for discriminating between hybrids classified as resistant or susceptible to PRR (**Figure 3**).

Figure 4

Field collection of a chemical fingerprint directly from an intact leaf using a handheld near-IR spectrometer. Photo by Enrico Bonello.

While these results are encouraging, further testing and validation are needed before the method is ready to be deployed as a tool for reliable identification of susceptible or resistant hybrid chestnuts. The accuracy of chemical fingerprint-based predictions may also be improved by using other types of predictive modeling, such as machine learning. Furthermore, handheld spectrometers are available and may be useful not only for identifying disease resistant trees but also for identifying diseased plants. In the latter case, near-IR spectroscopy shows great promise, as it is relatively non-invasive, requires minimal to no sample preparation, and chemical fingerprints can be collected in a matter of seconds (**Figure 4**).

For greater detail on these experiments, search for Anna's report from her 2015-2016 External Grant funded by TACF, available here: acf.org/resources/external-grants/

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