

SPECIES-DEPENDENT DESIGNED ULTRASONIC ACOUSTIC EMISSIONS OF TOMATO AND TOBACCO UNDER DROUGHT AND MECHANICAL INJURY: A PLANT-LEVEL CROSS-SPECIES ANALYSIS WITH AN EXPLORATORY VISUALISATION FRAMEWORK

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Abstract

Ultrasonic emissions offer a non-destructive route for detecting plant stress, but their transferability across species remains uncertain. A secondary analysis of 5,477 recordings from tomato and tobacco was conducted using 3,063 plant-emission events grouped into 170 plant-condition units representing drought and mechanical injury. Twenty-two temporal, amplitude and spectral descriptors were extracted and aggregated at plant level to prevent repeated emissions from the same plant entering both training and testing sets. Significant species × treatment interactions occurred for peak amplitude, root mean square amplitude and signal energy. Within-species classification achieved balanced accuracies of 0.747 for tomato and 0.706 for tobacco, whereas strict transfer was near chance from tomato to tobacco (0.518) and tobacco to tomato (0.484). Permutation analysis identified amplitude, dominant frequency, bandwidth and ultrasonic band energy as influential variables. The findings demonstrate measurable but strongly species-dependent acoustic stress signatures.

Introduction

Drought is a major limitation to plant growth and productivity because it alters water relations, stomatal behaviour, photosynthesis and carbon allocation. Mechanical injury represents a biologically different disturbance, but it also changes tissue integrity and initiates rapid local and systemic signaling. Conventional assessment commonly depends on visible symptoms, destructive measurements or instrumentation that is difficult to deploy continuously. Non-destructive sensing is therefore increasingly important for detecting stress before irreversible injury develops (Grossiord *et al.* 2020, Wahab *et al.* 2022, Zandalinas and Mittler 2022, Guadarrama-Escobar *et al.* 2024, Jiang *et al.* 2025).

Acoustic emissions provide one possible sensing channel. Drought-related ultrasonic events have been associated with hydraulic strain and cavitation processes, although airborne emissions may also reflect other passive physical changes in plant tissues (De Roo *et al.* 2016, Nardini *et al.* 2024). Mechanical damage activates complex wound responses involving hydraulic changes, glutamate receptor-like channels and calcium-based systemic signals, making it plausible that cutting and progressive water deficit produce different acoustic patterns (Toyota *et al.* 2018, Grenzi *et al.* 2023, Simon *et al.* 2023, Li *et al.* 2025).

The demonstration that tomato and tobacco plants emit airborne ultrasonic sounds under dehydration and cutting substantially expanded interest in plant bioacoustics. Those signals were sufficiently informative for computational classification and could be detected at a distance (Khait *et al.* 2023). Subsequent commentaries highlighted both the potential value of acoustic monitoring

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and the need for cautious interpretation, especially because evidence for universal plant communication through airborne sound remains incomplete (Hussain *et al.* 2023, Robert 2023, Waqas *et al.* 2023, Son *et al.* 2024). Recent work has also shown that lower-cost and outdoor recording systems are technically feasible, although environmental noise and signal standardization remain important challenges (Bonisoli *et al.* 2024, 2025).

Two methodological questions remain central. First, multiple acoustic events can originate from the same plant. Random allocation of such events to training and testing sets violates independence and may inflate apparent accuracy. Grouped or blocked validation is recommended when biological observations have hierarchical structure (Roberts *et al.* 2017). Second, successful classification within a mixed-species dataset does not establish that a stress signature is transferable between species. A model may instead rely on species-specific anatomy, amplitude scaling or frequency structure.

The present study re-analyzed the public tomato and tobacco acoustic archive at the plant-condition level. The objectives were to: (i) audit the recording archive and define the biological grouping unit; (ii) quantify species, treatment and species $\times$ treatment effects on ultrasonic features; (iii) classify drought and mechanical injury separately within tomato and tobacco; (iv) test strict cross-species transfer; (v) identify influential acoustic variables; and (vi) organize the outputs within an exploratory visualization framework. The working hypothesis was that drought and mechanical injury would be distinguishable within each species, but that the associated feature patterns would not transfer reliably between species.

Materials and Methods

The study used the public recordings released with the investigation of airborne sounds emitted by stressed plants (Khait *et al.* 2023). The archive comprised Tomato (*Solanum lycopersicum* L.) Dry, Tomato Cut, Tobacco (*Nicotiana tabacum* L.) Dry, Tobacco Cut, Empty Pot and Greenhouse Noises folders. Each waveform contained 1,001 samples recorded at 500 kHz, corresponding to 2.002 ms. In the original experiment, drought-treated plants had been deprived of water until severe soil-water deficit developed, whereas cut plants were recorded after mechanical severing. Empty-pot and greenhouse files were treated as environmental backgrounds, not as healthy-plant controls.

All WAV files were checked for readability, sampling rate, duration and exact waveform duplication. Labels were parsed from folder names. Filename patterns were used to extract plant and sound identifiers, and a plant-condition group was formed by combining class and plant identifier. The archive contained 5,477 valid recordings with no read errors or exact duplicates. The primary botanical analysis retained 3,063 plant emissions from 170 plant-condition groups. All events from the same group remained together conceptually, and the plant-condition group was treated as the biological unit (Table 1).

Stereo recordings, if present, were averaged to mono. Each waveform was converted to floating-point form and centred by subtracting its mean. Time-domain descriptors included root mean square amplitude, absolute peak amplitude, signal energy, crest factor, zero-crossing rate, active fraction above 20% of the peak, and rise and decay times relative to the maximum absolute amplitude. A Hann window was applied before the real fast Fourier transform. Spectral analysis was restricted to 20-120 kHz and included dominant frequency, spectral centroid, bandwidth, entropy, flatness, 85% roll-off, skewness, kurtosis, the proportion of total non-direct-current power within 20-120 kHz, and relative energy in five 20-kHz bands.

Event-level values were aggregated by the median within each plant-condition group. Interquartile ranges were retained for sensitivity checks but the primary models used the 22 median features. Each feature was rank transformed and analyzed using a two-factor model

containing species, treatment and species $\times$ treatment interaction. Type II sums of squares were used to derive F values and partial eta squared. P values were adjusted separately by model term using the Benjamini-Hochberg false-discovery-rate procedure. Within-species contrasts were evaluated using two-sided Mann-Whitney U tests with false-discovery-rate correction and Cliff's delta.

Logistic regression, radial-basis-function support vector machine and random forest models were evaluated. Logistic regression used $C = 1.0$, balanced class weights and a maximum of 5,000 iterations. The support vector machine used $C = 10$, gamma = scale and balanced class weights. The random forest used 150 trees, square-root feature sampling, a minimum leaf size of two and balanced subsampling. Standardization was fitted within each training fold for logistic regression and support vector machine. Plant-level performance was evaluated by five repeats of stratified five-fold cross-validation, yielding 25 test folds per model. Balanced accuracy was the primary metric, with macro-F1 and Matthews correlation coefficient as complementary measures. The reported 95% intervals are descriptive normal-approximation intervals across repeated folds.

For strict transfer, models were trained on all plant groups from one species and tested on all groups from the other species without target-species tuning. Species-specific standardized logistic coefficients were compared by Spearman correlation. Permutation importance was computed for the four-class random forest within five-fold validation, using balanced accuracy and eight permutations per fold (Fisher *et al.* 2019). A separate leakage check compared random clip-level splitting with stratified group splitting by plant-condition identifier.

The analytical workflow converted the recordings into linked visual outputs comprising representative waveforms, spectra and spectrograms; plant-level interaction plots; confusion matrices; transfer-performance displays; and feature-importance summaries (Fig. 1). The framework was designed for scientific interpretation of acoustic measurements. It was not evaluated as a user-interface or visual-design intervention.

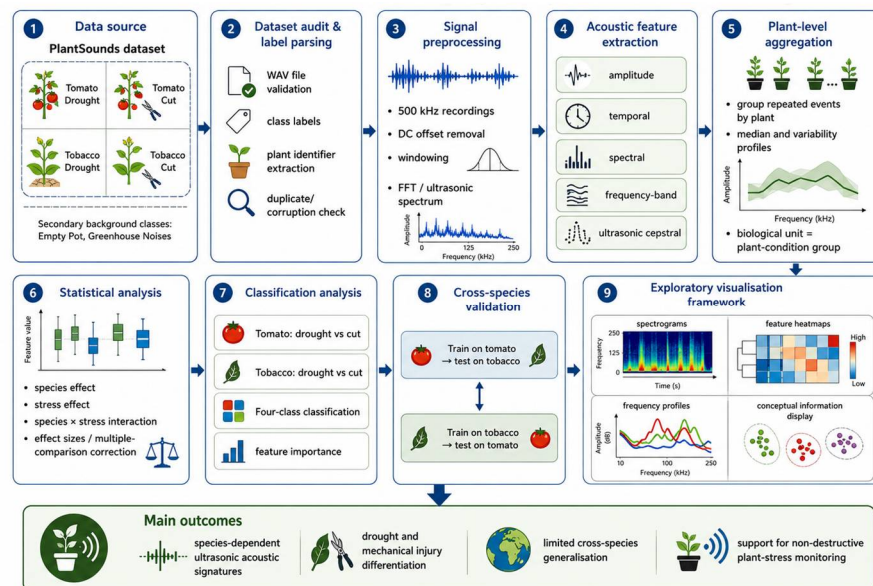


Fig. 1. Research architecture of the plant bioacoustics study. The workflow links dataset audit, signal processing, plant-level aggregation, factorial statistics, within-species classification, strict cross-species testing and exploratory visualisation.

The verified analysis was executed in Python 3.13.5 with a fixed random seed of 42. The full notebook, script, processed feature tables and out-of-fold prediction files were retained to support reproducibility. Missing or non-finite feature values were replaced by zero after feature extraction, consistently within the stored analysis pipeline.

Results and Discussion

The 5,477 readable recordings included 3,063 plant emissions and 2,414 environmental-background recordings. The plant-emission subset comprised 1,622 tomato drought events, 660 tomato mechanical-injury events, 275 tobacco drought events and 506 tobacco mechanical-injury events. These events represented 64, 43, 35 and 28 plant-condition groups, respectively (Table 1). The number of detected events per group ranged from 1 to 148, showing that sound events were highly clustered within plants. Consequently, event counts could not be interpreted as emission rates because equal recording duration per plant was not available. Representative traces nevertheless showed that the four classes contained distinct combinations of amplitude bursts and ultrasonic spectral energy (Fig. 2).

Table 1. Composition of the plant-emission dataset and plant-condition grouping used for the primary analysis.

Plant-treatment group	Sound events	Plant groups	Minimum events/group	Median events/group	Maximum events/group
Tobacco mechanical injury	506	28	1	17.5	65
Tobacco drought	275	35	1	7.0	27
Tomato mechanical injury	660	43	1	11.0	68
Tomato drought	1,622	64	1	7.5	148
Total	3,063	170	1	8.0	148

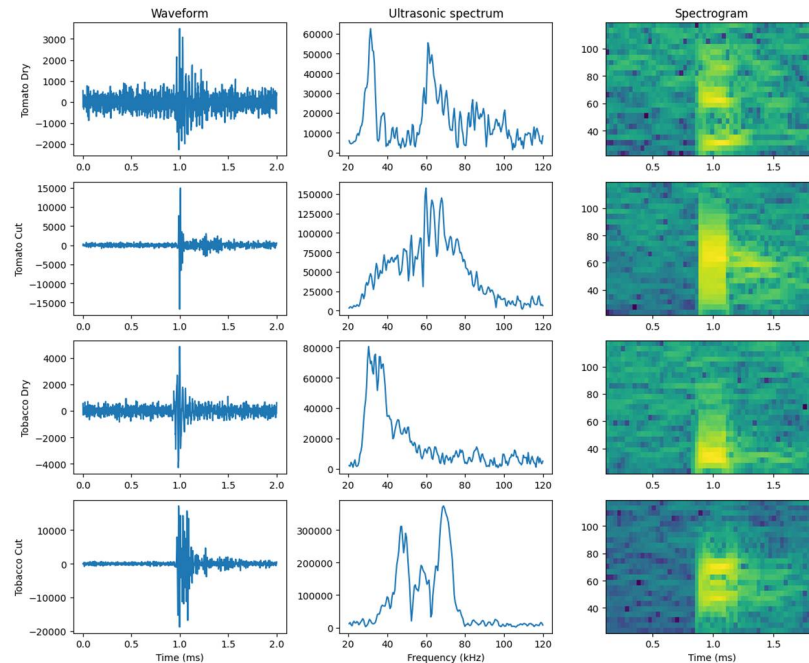


Fig. 2. Representative waveforms, ultrasonic spectra and spectrograms for tomato drought, tomato mechanical injury, tobacco drought and tobacco mechanical injury. Panels illustrate typical signal structure and are not estimates of class means.

Species-dependent treatment effects: Three amplitude-related variables showed significant species $\times$ treatment interactions after false-discovery-rate correction (Table 2, Fig. 3). Peak amplitude produced the strongest interaction ($F = 19.996$, adjusted $p = 0.000287$, partial eta squared = 0.108). The median peak amplitude increased from 6,915 in drought-treated tomato to 9,676 in mechanically injured tomato, whereas the tobacco contrast was smaller and reversed in direction. The within-tomato difference was large (Cliff's delta = -0.565, adjusted $p < 0.001$), but the tobacco contrast was not significant. Similar interactions occurred for root mean square amplitude ($F = 12.576$, adjusted $p = 0.00371$, partial eta squared = 0.070) and signal energy ($F = 12.395$, adjusted $p = 0.00371$, partial eta squared = 0.069). These results indicate that the acoustic expression of drought and injury was conditional on species rather than a uniform treatment response.

Table 2. Rank-based species $\times$ treatment interactions for the three amplitude-related plant-level features that remained significant after false-discovery-rate correction.

Feature	Tomato drought	Tomato injury	Tobacco drought	Tobacco injury	F	FDR-adjusted p	Partial eta squared
Peak amplitude	6,915	9,676	8,864	8,042	19.996	0.000287	0.108
RMS amplitude	695	928	904	821	12.576	0.00371	0.070
Signal energy	4.84×10^{-8}	8.66×10^{-8}	8.32×10^{-8}	6.91×10^{-8}	12.395	0.00371	0.069

Values in the first four columns are group medians.

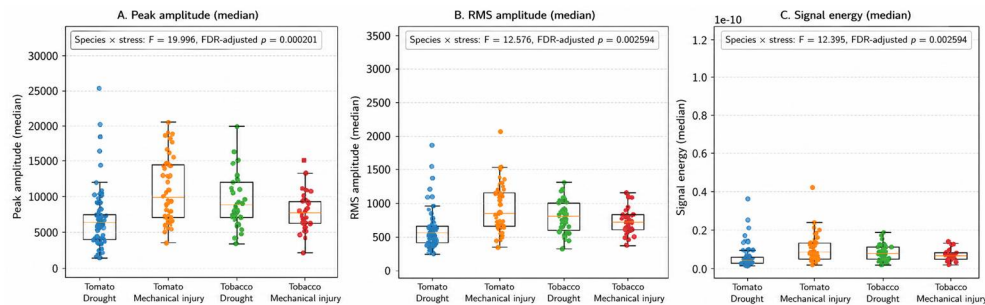


Fig. 3. Species $\times$ treatment interaction plots for peak amplitude, RMS amplitude and signal energy. Points represent plant-condition groups and boxes summarise the distributions. All three interactions remained significant after false-discovery-rate correction.

The statistical pattern is biologically plausible because drought and cutting disturb plant structure through different temporal processes. Progressive dehydration alters tension and water transport, while mechanical injury abruptly disrupts tissues and initiates hydraulic, electrical and chemical wound signals (De Roo *et al.* 2016, Toyota *et al.* 2018, Grenzi *et al.* 2023, Simon *et al.* 2023). However, the present secondary analysis did not include xylem pressure, water potential, gas exchange or anatomical measurements. The interactions therefore support species-dependent acoustic association, but they do not identify a unique sound-generation mechanism.

Plant-level acoustic profiles separated drought and mechanical injury with moderate performance. In tomato, the random forest was the best model, with balanced accuracy of 0.747, macro-F1 of 0.746 and Matthews correlation coefficient (MCC) of 0.515. In tobacco, the radial-basis-function support vector machine performed best, with balanced accuracy of 0.706, macro-F1 of 0.696 and MCC of 0.425 (Table 3, Fig. 4). The descriptive intervals remained above the binary chance level for both species. These results confirm that the aggregated ultrasonic variables

contain treatment-related information within species, although the scores are insufficient for claiming operational diagnosis.

Table 3. Best verified classification results at plant level.

Task	Best model	Balanced accuracy	95% interval	Macro-F1	MCC	Chance
Tomato drought vs injury	Random forest	0.747	0.712-0.782	0.746	0.515	0.50
Tobacco drought vs injury	RBF SVM	0.706	0.664-0.747	0.696	0.425	0.50
Four plant-treatment classes	RBF SVM	0.498	0.462-0.535	0.475	0.339	0.25
Train tomato, test tobacco	RBF SVM	0.518	-	0.507	0.036	0.50
Train tobacco, test tomato	Random forest	0.484	-	0.425	-0.050	0.50

Repeated-cross-validation intervals are descriptive 95% intervals across 25 test folds; strict transfer results are independent target-species point estimates.

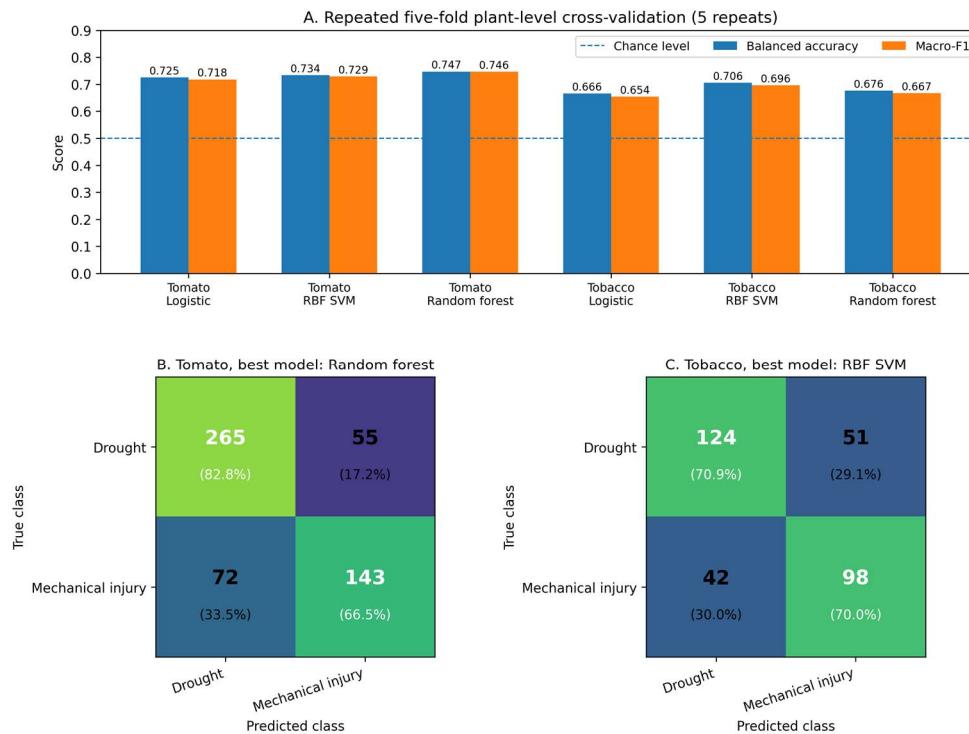
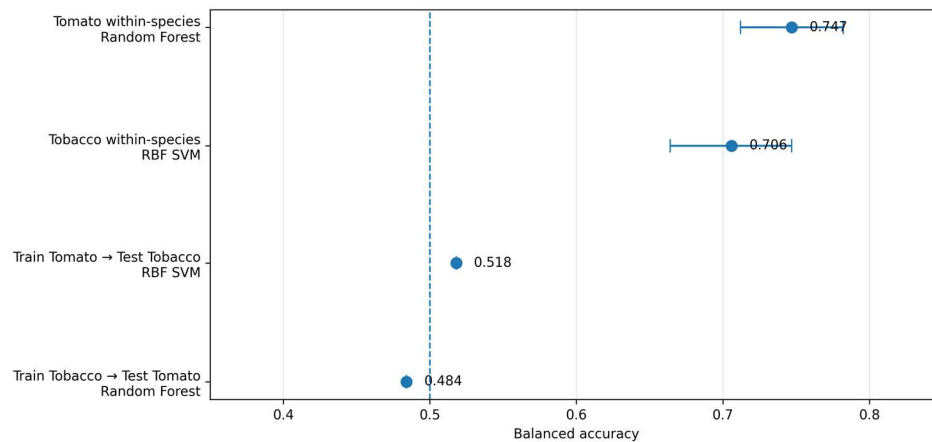


Fig. 4. Within-species model performance and confusion matrices. Bars show mean repeated five-fold cross-validation scores. Confusion matrices pool all repeated out-of-fold predictions, with each plant-condition group contributing one test prediction per repeat. The dashed reference indicates binary chance performance.

The joint four-class task was more difficult. The best model, the radial-basis-function support vector machine, achieved balanced accuracy of 0.498, macro-F1 of 0.475 and MCC of 0.339, compared with a four-class chance level of 0.25. The lower score relative to the binary tasks indicates overlapping species and treatment information. A majority-vote summary of repeated out-of-fold predictions gave a similar balanced accuracy of 0.503, but this value represents a different aggregation and was not used as the primary estimate.

Performance deteriorated sharply when the training and test species differed. Training on tomato and testing on tobacco produced a maximum balanced accuracy of 0.518 with the support vector machine. Training on tobacco and testing on tomato produced a maximum of 0.484 with the random forest (Table 3, Fig. 5). Both values were close to binary chance. In addition, the standardized stress coefficients estimated separately for the two species had a Spearman correlation of only 0.090 ($p = 0.689$). Thus, the variables associated with drought rather than mechanical injury in tomato did not show a consistent direction or magnitude in tobacco.



Error bars show descriptive 95% repeated-CV intervals for within-species evaluation. Cross-species results are strict external-transfer point estimates.

Fig. 5. Comparison of within-species classification and strict cross-species transfer. Error bars are descriptive repeated-cross-validation intervals for within-species estimates; transfer results are external target-species point estimates.

This finding refines the interpretation of earlier plant-sound classification research. The presence of informative sounds does not imply a species-independent acoustic code. Instead, classifier performance can depend on species-specific anatomy, baseline amplitude, spectral transmission or the severity and timing of stress. The present evidence therefore supports within-species acoustic phenotyping while arguing against immediate transfer of a model between tomato and tobacco. This cautious interpretation is consistent with recent discussions that recognise the value of plant acoustic emissions but distinguish detection from evidence of universal communication (Hussain *et al.* 2023, Robert 2023, Waqas *et al.* 2023, Nardini *et al.* 2024, Son *et al.* 2024).

Permutation analysis showed that the four-class model relied on both amplitude and spectral structure (Fig. 6). The strongest variables were peak amplitude, dominant frequency, spectral bandwidth, relative energy at 100-120 kHz, spectral centroid, relative energy at 20-40 kHz, signal energy and spectral skewness. Feature-family analysis further showed that tomato discrimination retained much of its performance using amplitude variables alone, whereas tobacco discrimination was close to chance with amplitude-only features and benefited more from frequency-band information. This contrast provides an additional explanation for poor cross-species transfer.

Leakage-aware interpretation: Random clip-level splitting yielded balanced accuracy of 0.403 in the four-class event-level comparison, whereas strict plant-grouped splitting yielded 0.374. The numerical difference was modest, but it was consistently in the expected direction. Events from the same plant share source characteristics and should not be treated as independent test observations. Grouped validation is therefore essential for estimating generalization to unseen plant-condition units (Roberts *et al.* 2017).

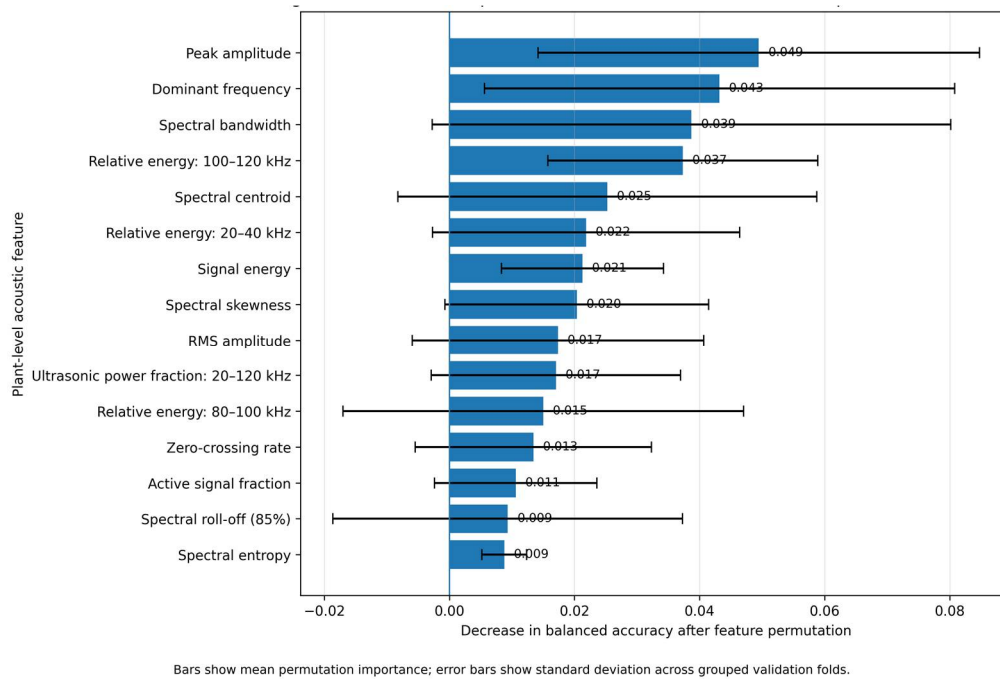


Fig. 6. Permutation importance of plant-level acoustic features for four-class classification. Bars show the mean decrease in balanced accuracy after feature permutation and error bars show variation across validation folds.

The visualization framework linked each analytical stage to an interpretable output. Waveforms displayed event timing and amplitude, spectra and spectrograms showed frequency organization, interaction plots described botanical effects, and performance and importance plots summarized predictive evidence. This presentation may support future monitoring systems, particularly as cost-effective and outdoor ultrasonic recording develops (Bonisoli *et al.* 2024, 2025). Nevertheless, the current framework is exploratory and was not tested for usability, information-transfer efficiency or decision-making speed.

Several limitations define the scope of the conclusions. The archive lacked a healthy, intact and well-watered plant class, and the empty-pot and greenhouse recordings cannot substitute for such controls. Plant identity was inferred from filename structure. Event numbers differed greatly among plant-condition groups, and equal recording-duration metadata were unavailable. The analysis included only two species and no independent field cohort. Physiological measurements were not available to link individual emissions to cavitation, water potential or wound signaling. Finally, repeated-cross-validation intervals are descriptive because fold estimates are not fully independent.

Ultrasonic acoustic features differentiated drought and mechanical injury within tomato and tobacco when the plant-condition group was treated as the unit of analysis. Significant species $\times$ treatment interactions, near-chance cross-species transfer and weak coefficient concordance collectively showed that the acoustic signatures contained substantial species-dependent structure. Plant bioacoustics may therefore contribute to non-destructive stress phenotyping, but robust application will require healthy controls, standardized plant-level recording durations, physiological co-measurements, broader taxonomic coverage and independent field validation.

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