Video Article

Evaluating *Dryocosmus Kuriphilus*-induced Damage on *Castanea Sativa*

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Abstract

Dryocosmus kuriphilus Yasumatsu has become a major pest for *Castaneasativa* since its arrival in Europe. Its galling activity results in the formation of different gall types and prevents the development of normal shoots. Repeated and uncontrolled attacks cause, besides the production of galls and the attendant gall-related reduction in leaf area, progressive corruption of the branch architecture, including the death of branch parts, and an increase in dormant bud activation. Thus far, there have been few attempts to quantify branch architecture damage. Further, the different methods for assessing infestation degree (MAID) that have been developed focus only on the galls' presence and abundance.

Using the leaf area to sapwood area relationship as a green biomass indicator, we developed in a previous study a damage composite index (DCI) that takes into account the most important branch architectural features, allowing for realistic damage assessment during the entire epidemic process.

The aim of this study is to present this novel method and highlight differences in the damage description with respect to other broadly used indices. Results show how the DCI depicts branch damage better, especially during the epidemic peak, compared to MAID, which tend to underestimate it. We conclude by suggesting how to properly evaluate the overall impact of the pest by means of our composite damage index, the infestation degree using classic methods, and crown transparency evaluations.

Video Link

The video component of this article can be found at https://www.jove.com/video/57564/

Introduction

The chestnut gallwasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is the most significant global insect pest of the genus *Castanea*[1,2,3]. Through its repeated galling activity, it prevents and inhibits normal shoot development[4,5], causing a progressive reduction of leaf area and a consequent loss of tree green biomass and vigour[5,6], dormant bud reactivation[5] and an increase in gallwasp post-emergence branch mortality[7,8].

The European experience of the gallwasp epidemic shows that uncontrolled and repeated gallwasp attacks may induce a high level of crown corruption in Sweet chestnut (*Castanea sativa* Mill.). This can result in crown leaf area losses of up to 70% that are neither compensated for by substitutive foliage produced by the activation of dormant buds nor by building second flushes during the same vegetation period[5].

The only successful method to reduce the pest population and allow chestnut trees to recover is biological control through its natural antagonist the parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae)[9,10]. Once biological control through its natural enemy is achieved, the chestnut trees start to produce new healthy sprouts. If tree damage level is very high, this may occur starting from the terminal bud only, due to the fact that it is usually infestation-free because of its formation after gallwasp oviposition activity[4]. This implies a long recovery process before the whole tree crown is re-established[5].

In order to check the positive reaction of chestnut trees after biological control by *Torymus sinensis* is reached, and to verify the need for sylvicultural (pruning, thinning) intervention, forest managers and chestnut growers need a method for quick and reliable assessment of damage level and related branch architecture and leaf area evolution throughout the gallwasp epidemic from the initial infestation phase by the pest to recovery after biological control by its antagonist. Several methods for assessing gallwasp infestation degree (MAID) have been developed and used worldwide to date, such as measuring the proportion of attacked buds[11] or the average number of galls per bud[12]. MAID do not directly measure green biomass (*e.g.,* leaf area), reserve structures such as dormant buds, reaction structures (*e.g.,* reactivated dormant buds and second flushes), or previous year damage (*e.g.,* dead shoots) as major proxies of current tree vitality and vigour[6,13,14]. Moreover, most MAID are only based on the number of galls found on tree branches and underestimate real branch damage, especially during the peak of the pest epidemic (**Figure 1**).

In this paper, we describe the damage composite index (DCI) approach proposed by Gehring *et al.* 2018[5] that considers proxies of green biomass, reserves such as dormant bud, and tree reactions (dormant bud reactivation and second flushes), enabling a realistic, reliable, and reasonably rapid assessment of damage through all stages of an epidemic, especially when combined with the assessment effort optimization proposed by Gehring *et al.* 2017[15].

In particular, the objectives of this paper are 1) to give a detailed description of the field protocol, including the relevant branch features to be assessed, 2) to present the damage composite index formula, and 3) to propose an improved severity scale conversion for the DCI.

Protocol

1. Tree Selection and Assessment Design

1. If possible, identify the epidemic stage of the study area by determining the arrival years of *Dryocosmus kuriphilus* and *Torymus sinensis* and the *T. sinensis* parasitization rate by using reliable sources (*e.g.,* scientific publications, forest services, chestnut grove managers' knowledge).
    1. If no reliable sources are available, identify the four main epidemic stages (Early, Peak, Recovery, Biocontrol) by computing the *T. sinensis* parasitization rate combined with the field observations described below.
        1. Identify the stage as **early epidemic** when tree crowns show neither significant damage nor crown transparency, current year galls are rare, and *T. sinensis* parasitization is very low or negligible.
        2. Identify **epidemic peak** when tree crowns display a high degree of transparency, although dead branches are rare, and current year galls are abundant.
        3. Identify **prolonged epidemic peak** when current and previous years' galls are abundant (up to three years previous) and *T. sinensis* parasitization is still very low or absent. Tree crowns still display high transparency and additional damage is represented by the first evidence of dead branches in the crown.
        4. Identify **early recovery stage** when *T. sinensis* parasitization rate becomes greater than 75%[10]. Damage is still high but the number of current year galls decreases and some branches produce gall-free shoots, especially from the apical bud.
        5. Identify **recovery stage** when *T. sinensis* parasitization rate is permanently greater than 75%, current year galls are rare and usually limited to single trees only and most branches produce gall-free shoots. Past years' galls on older branches and dead branches due to *D. kuriphilus* attacks are still visible.
        6. Identify **fully recovered stage** when damage and galls (past and current year) are rare or absent and crowns are fully recovered. In severely damaged trees, some vestiges (*e.g.,* dead shoots or rotten past years' galls) of prior *D. kuriphilus* attacks can still be present inside the crown.
        NOTE: Supplemental File 1 shows exemplary tree crown pictures for each epidemic stage.

2. Observe chestnut trees in the whole area to visually estimate damage variability among and within trees. Damage variability is usually low during the early epidemic and the recovery stages (crowns are basically healthy) as well as during the epidemic peak (whole crowns are full of galls). In contrast, variability tends to be high in the intermediate epidemic stages, when dead shoots due to past *D. kuriphilus* attacks are still present.
3. Based on 1.1 and 1.2, determine the number of trees to analyze. Unfortunately, it is not possible or suitable to give a specific rule regarding sample size, which may vary according to the specific epidemic situation in the field and/or the research objectives. Based on our 10 years of experience, for a 10-hectare site we advise the following (also see **Table 1** for details):
    1. Sample at least ten trees per site, regardless of the epidemic stage. Although during the early epidemic and recovered stage three trees would be enough, increasing the sample size to ten will give more statistical power to the results.
    2. During the early epidemic and recovered stage, sample one branch per tree.
    3. During the epidemic peak, sample one branch per tree if galls are evenly distributed within the tree crown, or two branches per tree if you notice that some crown parts have been attacked more severely.
    4. During the other epidemic stages, increase the number of branches to two (for trees that are recovering well) or three (for more damaged trees) based on the variability of crown damage of every tree.

2. Data Collection in the Field

1. Prepare the appropriate equipment including a clipboard, a camping chair, secateurs, a telescopic tree pruner, a 30 m measuring tape, and tree climbing equipment if the top crown above 8 m requires analysis.
2. Select the most representative branches trying to proportionally cover branch diversity within the tree crown (healthy trees usually have similar branches while damaged trees may have branches with different degrees of damage). For example, if you choose to collect three branches per tree, collect the most damaged branch, the healthiest and an intermediate one.
3. Whenever possible, select architectural branches only, while avoiding reiterations (trunk suckers or reiterations *sensu* Hallé)[16].
4. Ensure branches are at least 50 cm in length and have at least 10 shoots.
5. Attach the beginning of the measuring tape near the blade of the telescopic tree pruner in order to measure the height above ground of the branch at the cutting point.
6. Cut the branch with the telescopic pruner, record its cutting height, its aspect, its type (architectural or reiteration) and refine the branch selection with secateurs in order to keep only the part for analysis.
7. Assign a unique ID to the branch and record its age, its maximum length (from the first branching point to the apex) for general information.

8. Take a quick look at the whole branch to obtain a first impression of its history and present status (heavily attacked or not) and identify all the elements and features important for the calculation of the DCI with the help of **Figure 2** and **Figure 3**.

3. Branch Feature Definition

The following definitions are partially or totally reproduced from Gehring *et al.* 2018[5], with the permission of Springer-Verlag Berlin Heidelberg 2017.

1. Define a **Sprout** (on a shoot) as a freshly formed sprout that has grown during the current vegetative season from a developed bud on a shoot.
2. Define a **Shoot** as a sprout from the previous vegetative season with respect to the sampling date (*e.g.,* sampling season = 2017, shoot = sprout that grew in 2016). Can be dead or alive.
3. Define a **Dead shoot** (Sd) as a dead shoot after *D. kuriphilus attack* or due to natural death.
4. Define an **Alive shoot** (As) as a living shoot, not to be confused with a reactivated dormant bud.
5. Define a **Reactivated dormant bud** (Bdor) as a freshly formed sprout that has grown during the current vegetative season from a dormant bud on a multiyear branch part that is older than the shoot.
6. Define a **Gall on shoot** (Gons) as a gall developed at the base or along the axis of a sprout. Technically, these should be called "galls on sprouts" but for the purpose of consistency with existing literature, we refer to them as "galls on shoots".
   NOTE: **Figure 2** and **Figure 3** show examples of selected branch features. More detailed and complete descriptions (which are beyond the scope of this paper) may be found in Gehring *et al.* 2018[5] and Maltoni *et al.* 2012[4].

4. Branch Analysis

1. Count and record all the living shoots (alive shoots).
2. Count and record all the dead shoots.
3. Count and record all the reactivated dormant buds.
4. Count and record all the galls on shoots.
   NOTE: Supplemental File 2 shows an example of a field sampling form and Supplemental File 3 shows the sampling form filled out.

5. Calculation of the Damage Composite Index

1. Calculate the proportion of dead shoots (Sd) from the number of dead shoots divided by the total number of shoots (dead shoots + alive shoots).
2. Calculate the proportion of reactivated dormant buds (BdoR) from the number of reactivated dormant buds divided by the total number of living shoots (BdoR + alive shoots).
3. Calculate the average number of galls on shoots (Gons) from the number of galls on shoots divided by the number of living shoots (BdoR + alive shoots).
4. Calculate the DCI using the formula DCI = (Sd * 0.479 + BdoR * 0.525 + Gons * 0.120) * 100.
5. Use **Table 2** to evaluate the damage severity.
   Note: An R script with the DCI function is available in Supplemental Coding File 1. Its description is found in Supplemental File 4.

Representative Results

A total of 25 localities in Ticino, Switzerland were visited between 2013 and 2016 in order to create a temporal gradient covering all gall wasp epidemic stages. In total, we collected and analyzed 94 branches in 5 sites at an early epidemic stage (arrival of the pest and beginning of tree damage), 200 branches in 5 sites at the epidemic peak (medium to severe damage due to high level of *D. kuriphilus* attack), 200 branches in 5 sites at the recovery stage (biocontrol by *T. sinensis* reached and start of progressive tree recovery), and 54 branches in 5 sites where the pest returned at a very low level over the past 4-5 years. The DCI and two MAID ("average number of galls per bud" (GB)[12] and the "proportion of attacked buds" (AB)[11]) were calculated for each branch. Since the three indices are on different scales, a standardized severity scale from 1 to 9 (1 = very low damage, 9 = extreme damage) is used in order to compare them. DCI is rescaled according to Gehring *et al.* 2018[5], GB according to Sartor *et al.* 2015[12] and AB according to Gyoutoku and Uemura 1985[17](**Table 2**). Comparisons of DCI and MAID within branches were made using the nonparametric Wilcoxon signed-rank test with the Holm adjustment for *p* values.

At early stages of the epidemic, DCI and MAID are low (median values at 25^{th} and 75^{th} percentiles, DCI = 1 *[1, 1]*, AB = 1 *[1, 2]*, GB = 1 *[1, 2]*) and express the same level of damage ($p > 0.5$; **Figure 4**). During the epidemic peak, the DCI indicates very high damage levels (DCI = 8 *[7, 9]*) compared to both MAID that indicate only intermediate branch damage level (AB = 4 *[3, 5]*, GB = 4 *[3, 6]*; **Figure 4**). Considering branches with low GB and AB values (< 3), 34 out of 71 and 29 out of 59 have more than 30% dead shoots, respectively, whereas from branches with medium GB and AB values (4 and 5), 19 out of 76 and 30 out of 108 have more than 40% dead shoots, respectively. This objectively indicates a high degree of damage. During the recovery phase, differences between DCI and MAID are smaller but still significant ($p < 0.01$), whereas no differences persist when the recovered phase is reached. **Figure 5** provides a visual example of the possible causes of these dissimilarities during the different epidemic stages.

Generally speaking, during the epidemic peak, GB and AB expressed the same damage level as DCI in only 5% of the cases, while tending to underestimate it in ca. 85% of the cases. During the recovery phase, correspondence between GB and DCI and between AB and DCI occurred in 12% and 14% of the cases, respectively. Both MAID underestimated damage in 70% of the cases.

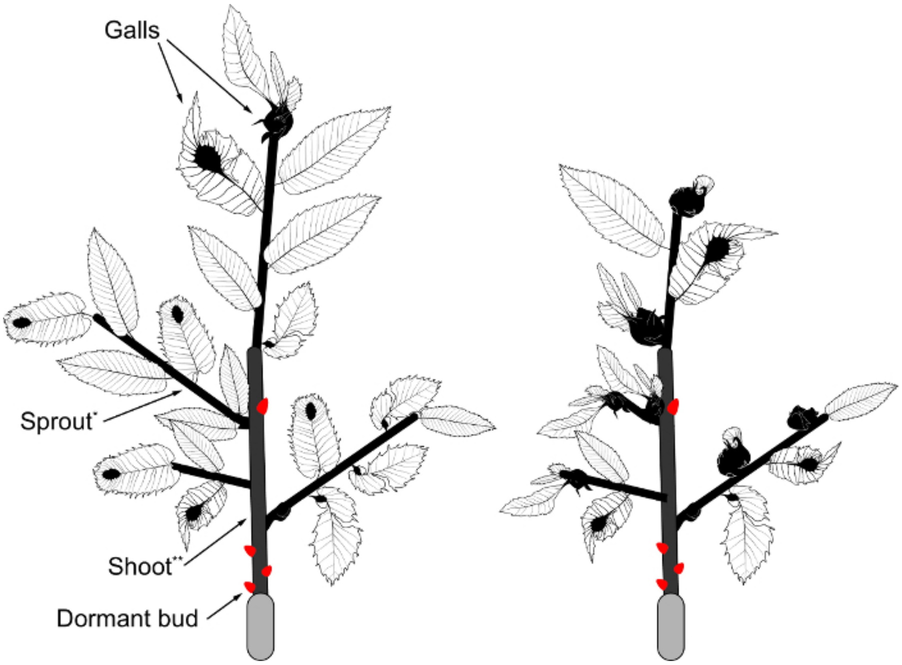

Figure 1: Branch damage evaluation illustrating the limitations of classic methods for assessing gallwasp infestation degree (MAID). The sketches show two branches with the same degree of GB (11 galls / 8 buds) and AB (4 attacked buds / 8 buds) but with very different leaf area losses. *Sprout: grown during the current vegetative season; **Shoot: sprout from the previous vegetative season. Please click here to view a larger version of this figure.

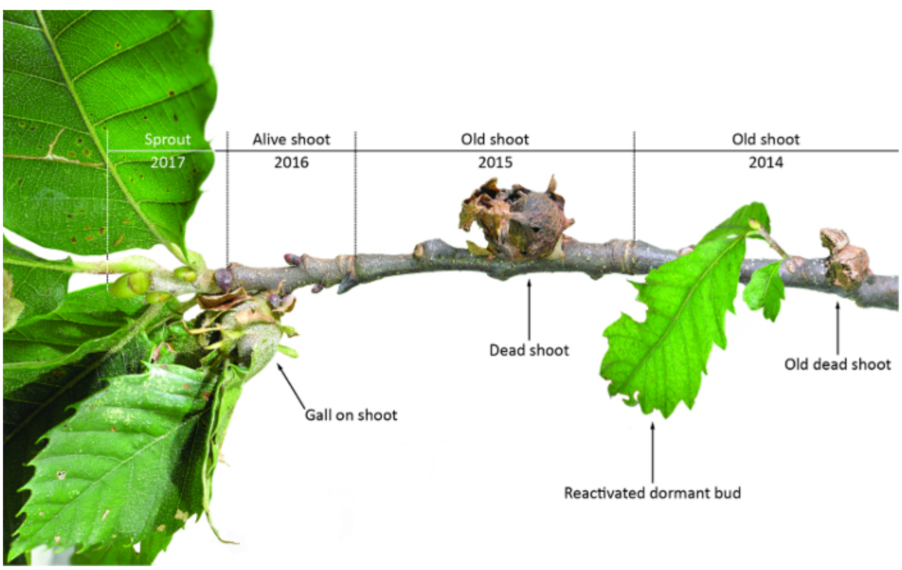

Figure 2: Main features considered when calculating the damage composite index. The dead shoot in the photo is technically a gall on shoot that grew in the previous vegetative season (2016) and completely prevented the elongation of the 2016 shoot. Because the entire gall is dead and no living buds are present on it, it is considered a dead shoot in 2017. Please click here to view a larger version of this figure.

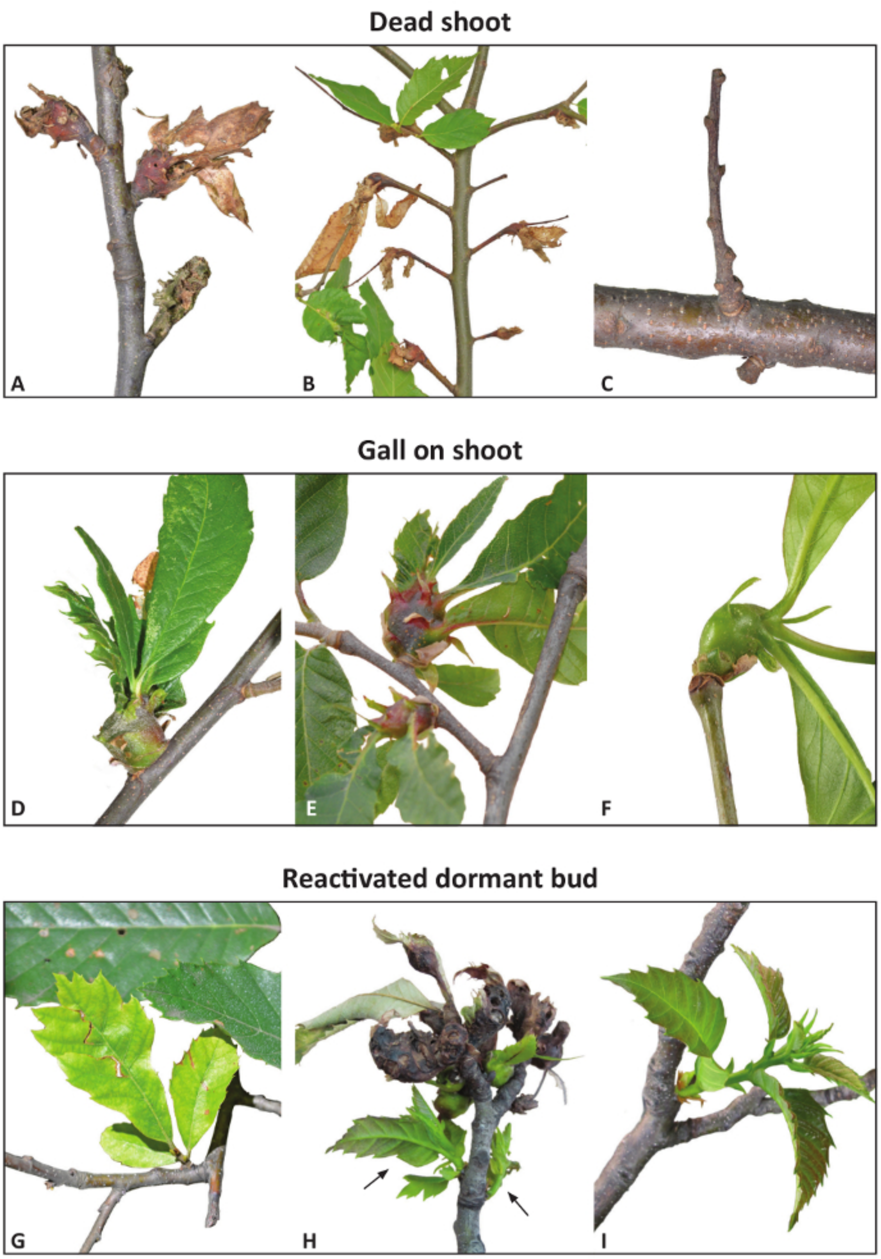

Figure 3: Essential branch features for assessing the damage composite index (DCI). The dead shoots in (A) and (B) are technically galls on shoots that grew during the previous vegetative season, completely prevented the elongation of the shoot, and are now dead. The dead shoot in (C) died for other causes. The rotten shoot at the bottom of (A) was already dead the year before the dead shoot present on that branch, and is consequently not considered in the DCI calculation. (D), (E) and (F) show different examples of galls on shoots, whereas (G), (H) and (I) represent reactivated dormant buds. Please click here to view a larger version of this figure.

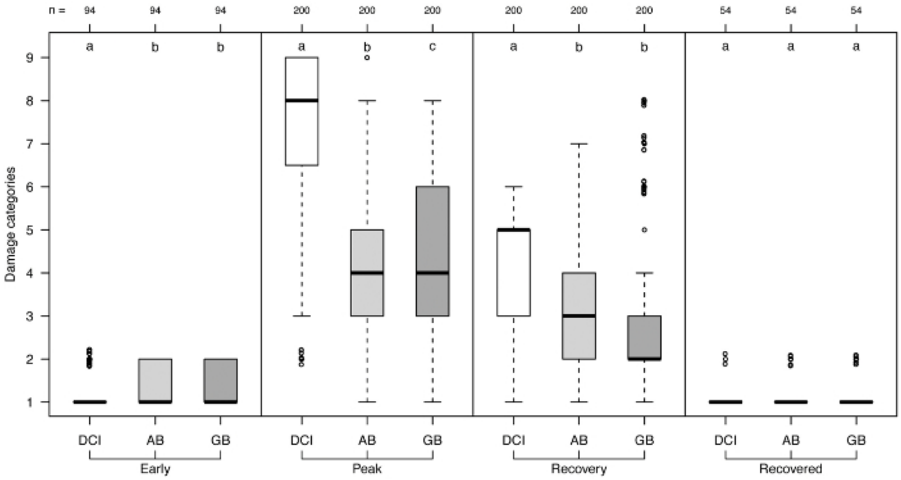

Figure 4: Evolution of the damage composite index (DCI) and two classic methods for assessing gallwasp infestation degree (MAIDs) across the different epidemic stages. Please refer to **Table 2** for more details on the damage categories. DCI: damage composite index (see paragraph 5 of the protocol); AB: proportion of attacked buds (no. attacked buds / no. buds); GB: average number of galls per bud (no. galls / no. buds). Labels on top (n) indicate the number of sampled branches per epidemic stage. Different letters indicate significant differences ($p < 0.01$) between DCI and MAID branch values at epidemic stages based on the nonparametric Wilcoxon signed-rank test with the Holm adjustment for *p* values. Outliers are defined as any observation falling outside 1.5 times the interquartile range above or below the upper or lower quartile respectively. Please note that noise has been added to outlier data to avoid excessive overlapping. Please click here to view a larger version of this figure.

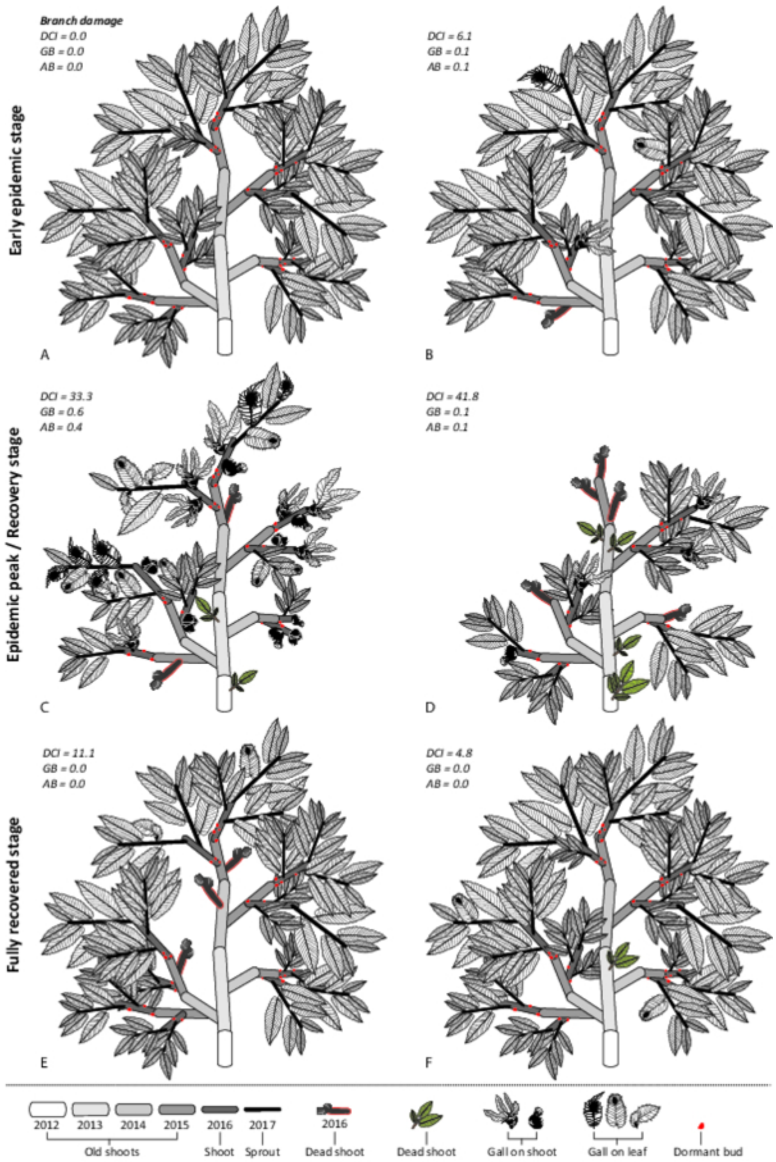

Figure 5: Schematic depictions of chestnut branches at different *Dryocosmus kuriphilus* epidemic stages. At early epidemic stages (A, B), branch architecture is still intact and both the damage composite index (DCI) and MAID (GB and AB) have very low values. Especially during the epidemic peak and recovery stages, branch architecture may become heterogeneously corrupted with different types and degrees of damage. The damage expressed by DCI, GB, and AB can thus be similar (C) or completely different (D) depending on the severity of branch corruption. Finally, at the recovered stage (E, F), DCI and MAID again have similarly low values with the DCI being somewhat more sensitive to previous year damage (dead branches). Please click here to view a larger version of this figure.

Epidemic stage	Years since arrival		Ts%	Tree	Branch
	Dk	Ts	%	n. per site	n. per tree
Early	1-2	0-1	0-5	10	1
Peak	3	2-3	>5 <70	10	1-2
Prolonged peak	4-5	2-3	>5 <30	10	2-3
Early recovery	6-7	4-5	>70	10	2-3
Recovery	8-10	6-8	>70	10	2-3
Fully recovered	>10	>9	>70	10	1

Table 1: Minimum number of trees and branches required based on epidemic stage and *Torymus sinensis* parasitization rate. Dk = *Dryocosmsus kuriphilus*; Ts = *Torymus sinensis*; Ts% = *Torymus sinensis* parasitization rate calculated as follows: number of living *T. sinensis* / total number of chambers * 100 (at gall level).

Conversion scale		DCI	GB	AB
Damage severity	Points		Average per shoot	Proportion
No damage	1	≤2.5	≤0.1	≤0.1
Very low	2	>2.5 - ≤5	>0.1 - ≤0.2	>0.1 - ≤0.2
Low	3	>5 - ≤7.5	>0.2 - ≤0.3	>0.2 - ≤0.3
Mild	4	>7.5 - ≤10	>0.3 - ≤0.4	>0.3 - ≤0.4
Moderate	5	>10 - ≤15	>0.4 - ≤0.5	>0.4 - ≤0.5
High	6	>15 - ≤20	>0.5 - ≤0.6	>0.5 - ≤0.6
Very High	7	>20 - ≤25	>0.6 - ≤0.7	>0.6 - ≤0.7
Extreme	8	>25 - ≤30	>0.7 - ≤0.8	>0.7 - ≤0.8
	9	>30	>0.8	>0.8

Table 2: Conversion scale for the three indices: damage composite index (DCI), number of galls per bud (GB) and attacked buds (AB). DCI is rescaled according to Gehring *et al.* 2018[5], GB according to Sartor *et al.* 2015[12], and AB according to Gyoutoku and Uemura 1985[17].

Supplemental File 1. Please click here to download this file.

Supplemental File 2. Please click here to download this file.

Supplemental File 3. Please click here to download this file.

Supplemental File 4. Please click here to download this file.

Supplemental Coding File 1. Please click here to download this file.

Discussion

Dryocosmus kuriphilus lay eggs in chestnut tree buds, inducing the formation of galls in spring. Repeated and uncontrolled *D. kuriphilus* attacks cause, in addition to gall formation, general branch corruption, including the death of many shoots and a significant loss in green photosynthetic leaf area[5]. Trees usually react by attempting to produce substitutive shoots via the activation of dormant buds. For this reason, especially during the epidemic peak and the recovery stage, classic MAID (based on galls abundance only) tend to underestimate the real damage caused by *D. kuriphilus* while the DCI, which is based not only on gall abundance but also on dead shoots and reactivated dormant buds, better reflects real damage severity and the degree of branch architecture alteration. In fact, MAID focus more on the population level of *D. kuriphilus* rather than on the degree of damage to trees. For example, when the branch damage is low and the presence of galls is insignificant during the early epidemic and the final, recovered stage, both MAID and DCI show very low values. However, during the epidemic peak or when exposed to several years of *D.kuriphilus* attack, a severely damaged branch may display many dead parts but only a few galls, or none at all. Using the criteria for each index, this would result in high DCI values (severe damage) but low MAID values (low damage).

It is thus important to understand the meaning and related damage degree indicated by each index in order to select the most suitable pest assessment approach according to the desired purpose. We therefore suggest evaluating the overall impact of the pest by using the DCI for the assessment of branch architecture damage, especially during the epidemic peak and the recovery stage. To ensure thorough pest assessment, classic MAID may be used to evaluate pest population levels (we suggest Gehring *et al.* 2017[15] for advice on how to adapt the sampling effort) whereas DCI may be used for a detailed assessment of branch architecture as well as for the entire tree crown. For a simple general assessment of crown transparency, in contrast, a tree assessment using the SANASILVA crown transparency index[18] may be the most suitable in terms of effort-benefit balance.

Once the operator becomes familiar with the main branch structures and features needed, applying the DCI is quite simple and relatively fast. However, in case of highly damaged branches due to repeated *D. kuriphilus* attacks, it could be difficult to properly assess the number of dead shoots because of the presence of older dead branches. It is thus important to try to reconstruct the branch history and to evaluate the degree of rot in the dead branches and shoots. Usually, dead shoots are not rotten or are less so compared to dead branches.

At the tree level, the major difficulty when evaluating the average branch damage of a large tree with heterogeneous crown damage (*e.g.,* a tree displaying healthy, damaged, and dead branches) regards the quantity of branches for analysis and the tree climbing skills and effort needed to reach the top of the crown. Moreover, since the method is destructive, evaluating a damaged tree will inevitably inflict a temporary additional loss of green biomass. It is therefore important to only select the smallest number of most representative branches in order to avoid excessive cutting.

Deciding on the correct sample size can sometimes be tricky. Based on our experience we note that in both the early epidemic and the fully recovered stage, damage on trees is absent and, consequently, its variability is very low. Consequently, sampling 10 trees per site and 1 branch per tree already gives a fair estimate of the damage. In contrast, during the epidemic peak and the recovery stage, trees show different damage levels. A good balance between sampling effort, data accuracy, and tree damage infliction can be reached by collecting 3 branches per tree for a total of 10 trees per site. Please note that this advice is based on our experience and research needs. Others are free to increase or decrease sample sizes according to their specific situation and assessment goals.

Finally, it is possible that at the beginning of the recovered phase, when almost no galls or dead shoots are visible on branches any longer, the DCI (and MAID) underestimate the residual damage present on the branches. This residual damage is represented by the possible lack of lateral shoots and dormant buds that occur especially in severely damaged trees that have suffered from repeated *D. kuriphilus* attacks over the years[5]. The lack of lateral shoots implies that a part of the leaf area is still missing, whereas the lack of dormant buds indicates that the tree has still not fully recovered its reserves.

Future or other applications of the DCI are difficult to imagine because it is species-specific and the constants applied in its calculation are calibrated to the chestnut tree[5]. Nevertheless, the methodology used to create it could be adapted and implemented to any other tree species and related pest.

Disclosures

The authors have nothing to disclose.

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