

Article

Two Decades of Monitoring *Tortrix viridana* and Associated Oak-Defoliator Groups in the Republic of Moldova

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Abstract

Long-term phytosanitary monitoring provides essential information on temporal changes in forest-insect populations, but conclusions depend on consistent sampling and accurate definition of the taxa included in the analysis. We examined the dynamics of *Tortrix viridana* L. (Lepidoptera: Tortricidae) and two associated operational groups, other Tortricidae and Pyralidae, in *Quercus robur* L. stands at Moleşti–Răzeni and Tigheci–Cociulia in the Republic of Moldova. Monitoring covered the 2005–2025 calendar interval and provided 20 annual larval observations per site; larval data were unavailable for Moleşti–Răzeni in 2015 and for Tigheci–Cociulia in 2014 because of phytosanitary treatments. Annual larval densities showed pronounced interannual variability and distinct site-specific peaks. Other Tortricidae had the highest mean larval density at both sites, whereas *T. viridana* represented a smaller and temporally variable component of the three-group assemblage. After correction for temporal autocorrelation and multiple testing, significant decreasing trends in *T. viridana* and Pyralidae larval densities were detected at Moleşti–Răzeni, whereas no focal group showed a significant temporal trend at Tigheci–Cociulia. The results do not support a uniform regional decline of *T. viridana* or any other focal group. Instead, they demonstrate spatially heterogeneous population dynamics and show that operational forest surveillance should consider multiple defoliator groups rather than relying exclusively on *T. viridana*.

Keywords: *Quercus robur*; *Tortrix viridana*; Tortricidae; Pyralidae; forest insect monitoring; larval density; long-term population dynamics; phytosanitary surveillance



Academic Editors: Bakier Slawomir and Piotr Borowik

Received: 16 August 2026

Revised: 15 September 2026

Accepted: 17 September 2026

Published: 21 September 2026

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1. Introduction

Oak forests (*Quercus* spp.) represent one of the most ecologically and economically valuable forest ecosystems throughout Europe, ensuring numerous ecological functions including biodiversity conservation, climate regulation, carbon sequestration, hydrological stability, and maintenance of ecosystem services. In the Republic of Moldova, oak-dominated forests constitute a major component of the national forest fund and represent

the principal natural forest communities. *Quercus robur* L. occupies approximately 78,000 ha, accounting for nearly 24% of the total forest-covered area and approximately 55%–56% of all oak stands in the country [1].

The forest sector of the Republic of Moldova is characterized by a relatively low degree of forest cover and high fragmentation of forest resources. National forest cover represents approximately 12%–13% of the country's territory, with most forests occurring as isolated and discontinuous stands [1]. Uneven spatial distribution, anthropogenic pressure, and fragmentation reduce ecological connectivity and increase ecosystem vulnerability to environmental disturbances. Furthermore, the territory of the Republic of Moldova is situated at the intersection of three major biogeographical regions, the Central European zone, Eurasian forest-steppe, and Mediterranean xerophytic area, generating complex ecological conditions and high biodiversity [2].

Although ecological conditions are generally favorable for the development of productive oak stands, the present condition of Moldovan oak forests reflects a long history of intensive management. Most oak forests originated from coppice systems maintained over the last two or three centuries. According to Boaghi [3], more than 80% of oak stands were reported to be of sprout origin, many belonging to third–eighth vegetative generations. Long-term management under coppice regimes has reduced stand stability and biological resistance and has negatively affected reproductive potential and natural regeneration capacity.

In recent decades, oak ecosystems throughout Europe have experienced increasing pressure associated with multiple interacting stress factors [4–9]. Repeated droughts, climatic anomalies, late spring frosts, habitat degradation, and biotic disturbances have contributed to widespread reductions in oak vitality and productivity [5,10]. In many regions, oak decline has become an important ecological issue resulting from cumulative interactions between abiotic and biotic stressors, a trend observed not only in the Republic of Moldova but across numerous European countries [7,11]. Recent studies suggest that ongoing climatic changes may substantially alter forest insect dynamics and host–insect interactions, thereby increasing the risk of disturbance events and modifying the outbreak patterns of herbivorous insect species [12,13].

Biotic disturbances represent a major threat to the stability and resilience of oak ecosystems. Among these factors, defoliating insects play a critical role due to their capacity to alter physiological processes, reduce tree vigor, and affect ecosystem functioning. Recurrent defoliation events negatively affect photosynthetic activity, decrease annual increment, weaken host resistance mechanisms, and increase tree susceptibility to secondary pests and pathogens. Consequently, repeated insect attacks may contribute to long-term reductions in stand vitality [12,14]. In oak forests, defoliation is typically caused not by a single species but by a complex of phytophagous Lepidoptera, including Tortricidae, Pyralidae, and other associated groups [15–17]. Monitoring multiple groups within this pest complex provides a broader context for interpreting the population dynamics of key defoliators and supports more comprehensive forest-protection assessments.

Within the oak-associated defoliator complex, *Tortrix viridana* L. (Lepidoptera: Tortricidae), commonly known as the green oak leaf roller, is considered one of the most important early-season defoliating species in European oak forests [18–20]. The species exhibits a broad distribution extending across Europe, Western Asia, and North Africa and shows a strong ecological association with *Quercus* species. *T. viridana* is univoltine and overwinters as eggs deposited on branches and buds [19,20]. Larval emergence occurs in early spring and shows close synchronization with oak budburst, which is critical for successful development as first-instar larvae require newly opened buds and young foliage of high nutritional quality [21]. Even small phenological shifts between insect emergence and host

development may substantially influence larval survival and population success [22,23]. During outbreak phases, larval feeding intensity may increase dramatically, resulting in large-scale defoliation and substantial effects on oak health and stand functioning [24,25].

Because population outbreaks of *T. viridana* may cause extensive early-season defoliation of oak crowns, the species has historically received particular attention within the forest-protection programme implemented by the Forest Research and Management Institute (ICAS) in the Republic of Moldova. Its univoltine life cycle, overwintering egg stage, and generally recognisable early-instar larvae also made it suitable for repeated operational surveillance. Consequently, long-term monitoring included both spring larval assessments and species-specific counts of viable egg masses after oviposition. These observations were used to estimate population development and to support operational forecasts of potential crown defoliation and decisions regarding phytosanitary intervention. This historical monitoring framework explains the species-level emphasis placed on *T. viridana* in the present analysis, while the other consistently recorded defoliators were retained as broader operational groups [26].

Population fluctuations of *T. viridana* may exhibit cyclic patterns, with prolonged periods of low abundance alternating with episodic population eruptions [17,27,28]. The occurrence and severity of such outbreaks are closely associated with interactions among climatic conditions, host phenology, and ecological regulatory factors [10,14,18,19]. Climatic variability is considered one of the major drivers of long-term forest insect population dynamics [8,12]. Temperature affects developmental rates, survival, and emergence timing, while precipitation regimes may influence host vigor and habitat suitability [8,12,14,18]. Studies conducted in Ukraine demonstrated climate-induced shifts in *T. viridana* population dynamics, suggesting changes in outbreak frequency and temporal patterns under changing environmental conditions [17,27,28]. Pheromone-based studies have also demonstrated the utility of species-specific monitoring for assessing the seasonal activity of *T. viridana* [25].

Despite the ecological and economic importance of oak-associated defoliating insects, comprehensive long-term analyses of multiple components of the oak-defoliator complex, integrating population trends of multiple taxonomic groups with associations among operational taxonomic groups and site-level comparisons, remain limited in southeastern Europe, particularly for the Republic of Moldova [16,17]. Although valuable information is generated through annual phytosanitary monitoring conducted by the Forest Research and Management Institute (ICAS, Republic of Moldova), such datasets have rarely been synthesized within long-term ecological analyses.

Therefore, the aims of the present study were: (1) to characterize the long-term population dynamics of *T. viridana*, other Tortricidae, and Pyralidae in southern Moldova across the 2005–2025 monitoring interval; (2) to detect monotonic temporal trends in the larval densities of the three focal groups using non-parametric statistical methods; (3) to assess associations among the annual larval densities of the three focal groups; and (4) to compare paired annual population indicators between the two monitoring sites.

2. Materials and Methods

2.1. Study Area

The study was conducted in two pedunculate oak (*Quercus robur* L.) forest complexes located in the southern part of the Republic of Moldova. The first monitoring site was situated in the Moleşti–Răzeni forest (3333.1 ha), managed by the State Forestry and Hunting Enterprise Sil-Răzeni, within the Răzeni Forest District (46°52' N, 28°57' E). The second monitoring site, hereafter referred to as Tigheci–Cociulia, was located in the Codrii Tigheci II forest complex (2967.7 ha), administered by the Cociulia Forest District, part of the State Forestry Enterprise Silva-Sud Cahul (46°19' N, 28°24' E).

Both study sites are located within the forest-steppe zone of southern Moldova and are characterized by a temperate continental climate with warm, dry summers and moderately cold winters. The monitoring sites are approximately 74 km apart, with Tigheci–Cociulia situated farther south than Molești–Răzeni. Under the national forest classification applicable during most of the monitoring period, both forest complexes were classified as Group I forests designated for special protection functions (Figure 1) [29,30].

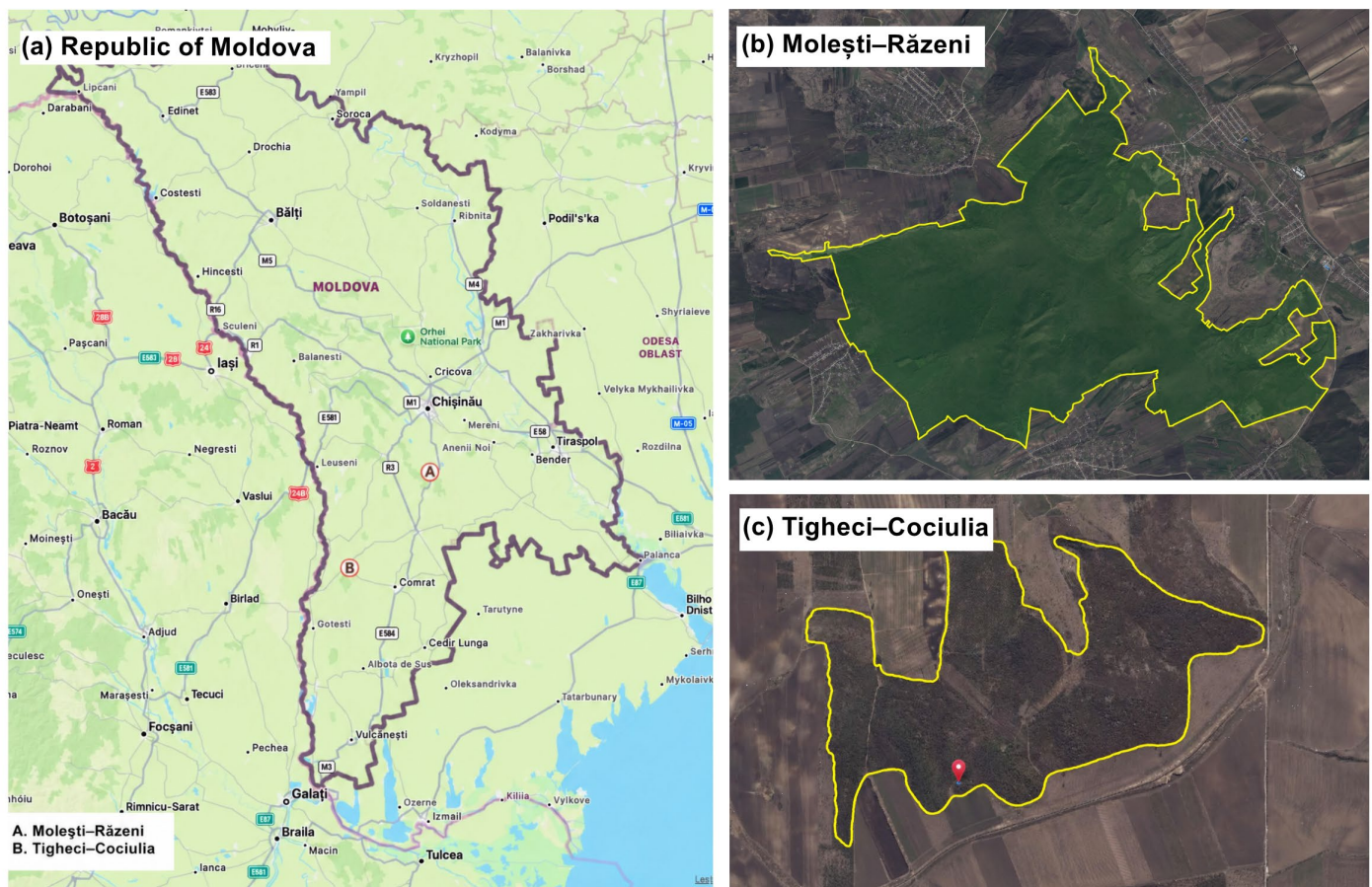


Figure 1. Geographic location of the two permanent oak-defoliator monitoring sites in the Republic of Moldova. (a) Regional position of the Molești–Răzeni (A) and Tigheci–Cociulia (B) monitoring sites; the thick dark-purple line delineates the national border of the Republic of Moldova. (b,c) Satellite views of the Molești–Răzeni and Tigheci–Cociulia forest complexes, respectively. Yellow lines indicate the approximate boundaries of the forest areas included in the long-term monitoring programme. The red location marker in panel (c) indicates the position of the Tigheci–Cociulia permanent monitoring site.

Forest stands at both monitoring sites are dominated by pedunculate oak (*Quercus robur* L.), with admixtures of *Carpinus betulus* L., *Fraxinus excelsior* L., and *Acer campestre* L. Stand age at Molești–Răzeni ranged between 60 and 80 years, while stands at Tigheci–Cociulia were generally older, reaching 90–120 years. Both sites are representative of the mature oak forest ecosystems in southern Moldova and have been included in long-term phytosanitary monitoring of foliage-feeding insects.

2.2. Population Monitoring

Long-term monitoring of *Tortrix viridana* L. and two associated defoliator groups, other Tortricidae and Pyralidae, covered the 2005–2025 calendar interval at both Molești–Răzeni and Tigheci–Cociulia. The resulting larval-density dataset comprised 20 annual

observations per site. Larval assessments were unavailable for Molești-Răzeni in 2015 and for Tigheci-Cociulia in 2014 because phytosanitary treatments were conducted during those years. Monitoring followed the standardized forest-pathological surveillance protocols applied by the Forest Research and Management Institute (ICAS) in the Republic of Moldova [26]. One larval assessment was conducted annually at each monitoring site during spring larval development, when early-instar defoliating larvae were present on newly developed oak foliage. At each monitoring site, three sampling blocks of 50 oak trees were established in stand areas with broadly comparable species composition, structure, stocking density, age, and terrain exposure. Three representative model trees were selected from each block for every annual larval assessment, resulting in nine model trees per site and year. Individual model trees were not permanently marked; they were selected at each assessment using the same standardized criteria, including stem diameter, tree height, and crown development. From each model tree, one branch extending from the middle part of the crown toward its periphery, generally on the southern exposure, was sampled during each survey. The sampled branch did not exceed approximately 3 cm in basal diameter. It was lowered onto a white collecting cloth, and larvae falling onto the cloth or remaining on the foliage were counted and identified. Branches and foliage were not weighed directly. Fresh foliage mass was estimated from branch basal diameter using an internal ICAS calibration applied consistently throughout the monitoring program and at both sites. Woody branch mass was not included in the estimate. Larval density was calculated for each sampled branch as the number of larvae standardized to 100 g of estimated fresh foliage mass and was expressed as larvae 100 g^{-1} . The annual site-level value was calculated from the standardized densities obtained for the nine sampled branches. The multiplication coefficient for larvae (K_{larv}) was calculated as the ratio between larval density recorded in consecutive years. The coefficient was not calculated when larval density in the preceding year was zero or when either of the two consecutive annual observations was unavailable.

One egg-mass assessment was conducted annually at each monitoring site after oviposition, during the survey period extending from July into late summer or autumn. From each model tree, one branch extending from the middle part of the crown toward its periphery was examined. The selected branch did not exceed approximately 3 cm in diameter and comprised at least 5 m of cumulative linear branch length. Current-year shoot growth was excluded from the measured branch length in accordance with the national surveillance protocol [26]. All viable egg masses deposited on sampled branches were counted manually, and branch length was measured. Egg mass density (D , egg masses m^{-1}) was calculated as $D = N/L$, where N is the number of viable egg masses recorded and L is the examined cumulative branch length (m). In addition to viable egg-mass density per meter of branch, the ICAS records included an ecological egg-mass density standardized to 100 g of estimated fresh foliage mass. These two egg-related indicators were retained and analysed separately. The multiplication coefficient for egg masses (K_{egg}) was calculated analogously. As for larval density, the coefficient was not calculated when the preceding annual value was zero or unavailable. Under the national ICAS surveillance methodology, a density of 30 viable egg masses m^{-1} of branch length represents an operational forecast of 100% potential crown defoliation, conditional on successful egg hatching and larval survival [26]. This value was used as a phytosanitary forecasting benchmark and was not independently validated in the present study.

Aerial phytosanitary treatments targeting the oak-defoliating Lepidoptera complex were applied during spring larval development, particularly against first- and second-instar larvae. Treatments were conducted at Molești-Răzeni in 2012 and 2015 and at Tigheci-Cociulia in 2014. Larval observations were available for Molești-Răzeni in 2012 but were unavailable for Molești-Răzeni in 2015 and for Tigheci-Cociulia in 2014. Because

detailed information on the active substances, application rates, and exact timing relative to sampling was unavailable, treatment effects were not tested statistically.

2.3. Defoliator Grouping and Relative Composition

For the present analysis, larval records were assigned to three focal operational groups: *T. viridana*, other Tortricidae, and Pyralidae. These groups were selected because they were the only defoliator categories recorded with sufficient continuity and comparable taxonomic resolution at both monitoring sites throughout the 2005–2025 interval. *T. viridana* was retained as a separate species because of its historical importance in oak forest protection, the availability of species-specific egg-mass monitoring and an operational forecasting threshold, and the generally reliable morphological identification of its early-instar larvae under field conditions. The remaining Tortricidae and Pyralidae were retained as family-level operational groups because their early-instar larvae could not be consistently resolved to species level during routine field surveillance. Larvae were identified morphologically in the field using external diagnostic characteristics and the identification literature routinely applied within the ICAS forest-protection programme [31–35]. Larvae were not reared in the laboratory for subsequent identification. Additional defoliators recorded during the surveys included *Operophtera brumata*, *Erannis defoliaria*, other Geometridae, Noctuidae, Tenthredinidae, *Lymantria dispar* at Tigheci–Cociulia, and other incompletely identified taxa. These records were not included in the primary relative-composition analysis because their taxonomic resolution and completeness were not sufficiently consistent across sites and years.

For each monitoring year and site, the relative contribution of each focal group was calculated as its larval density divided by the summed larval density of the three focal groups and multiplied by 100. These percentages therefore describe relative composition within the three-group assemblage and not within the complete oak-defoliator community. Because the three proportions sum to 100%, they were treated descriptively and were not subjected to independent trend or correlation tests. The three focal groups were monitored at both sites throughout the 2005–2025 calendar interval, yielding 20 years with complete larval observations per site.

2.4. Statistical Analysis

All statistical analyses were performed in R version 4.6.0 [36]. Descriptive statistics (mean, standard deviation, standard error, median, minimum, maximum, and coefficient of variation) were calculated for the available population indicators at each monitoring site. Missing observations were coded as NA and excluded pairwise from the corresponding analyses; they were not treated as zero densities.

Temporal trends in the absolute larval densities of *T. viridana*, other Tortricidae, and Pyralidae were assessed using the non-parametric Mann–Kendall trend test, implemented with the Kendall package version 2.2.2 [37]. This test is appropriate for monotonic trend detection in time series with non-normal distributions and is widely used in ecological studies with moderate sample sizes. Kendall's τ statistic and associated p -values are reported. Temporal autocorrelation was examined using lag-1 Spearman correlation of the detrended series, restricted to pairs of consecutive calendar years. When significant temporal autocorrelation was detected, the Hamed–Rao variance-corrected Mann–Kendall test was applied; otherwise, the standard Mann–Kendall test was retained. The magnitude of temporal change was estimated using the Theil–Sen slope and its 95% confidence interval.

The three larval-density trend tests conducted at each site were treated as one family of primary comparisons, and p -values were adjusted using the Benjamini–Hochberg false-discovery-rate procedure. Both raw and adjusted p -values are reported. Relative-

composition percentages were treated descriptively because the three proportions sum to 100% and are therefore compositionally constrained. The primary trend analyses included all available annual observations. Because larval data were available for Molești–Răzeni in the treatment year 2012, sensitivity analyses were also conducted after excluding this observation. Treatment years without larval assessments were retained as missing values and were not interpolated. Statistical significance was evaluated at an adjusted p -value of <0.05 . Exact raw and adjusted p -values are reported rather than significance symbols alone. Pairwise associations among the annual absolute larval densities of *T. viridana*, other Tortricidae, and Pyralidae were assessed using Spearman rank correlation coefficients, calculated with the base R `cor.test()` function. Analyses were conducted separately for each monitoring site using years with data available for both groups. Correlations between an individual group and a total containing that group were not calculated because such part–whole relationships are mathematically circular. Spearman's r_s is reported. Within each site, p -values for the three pairwise correlations were adjusted using the Benjamini–Hochberg procedure.

Differences between the two monitoring sites were assessed using the paired Wilcoxon signed-rank test because observations obtained in the same calendar year were naturally paired. For larval-density comparisons, only years with observations available at both sites were included (2005–2013 and 2016–2025; $n = 19$ paired years). Rank-biserial correlation was reported as an effect-size measure. p -values for comparisons of the three focal larval-density groups were adjusted using the Benjamini–Hochberg procedure. Data import and result export were performed using the `readxl` package version 1.5.0 [38] and the `openxlsx` package version 4.2.8.1 [39], respectively.

Claude (Opus 5, Anthropic, San Francisco, CA, USA) and ChatGPT (GPT-5.6, OpenAI, San Francisco, CA, USA) were used to assist with R code generation, revision, and statistical workflow verification. All analytical decisions, calculations, and outputs were independently checked by the authors.

3. Results

3.1. Long-Term Population Dynamics of *Tortrix viridana*

Monitoring at Molești–Răzeni across the 2005–2025 calendar interval provided 20 annual larval observations, with no larval assessment conducted in 2015. *Tortrix viridana* larval density showed pronounced interannual variability, with a mean of 2.89 ± 4.30 larvae 100 g^{-1} (CV = 149.0%) and values ranging from 0.21 larvae 100 g^{-1} in 2022 to 18.04 larvae 100 g^{-1} in 2012. The multiplication coefficient for larvae (K_{larv}) reached a maximum of 23.76 in 2009, indicating a marked increase relative to the preceding year. Ecological egg-mass density, standardized to 100 g of estimated fresh foliage mass, was available for all 21 years and averaged 5.72 ± 7.16 egg masses 100 g^{-1} (CV = 125.1%), with a maximum of 26.28 egg masses 100 g^{-1} in 2015. Viable egg-mass density per metre of branch was available for 11 years (2013–2023), averaging 1.53 ± 1.88 egg masses m^{-1} and reaching a maximum of 5.85 egg masses m^{-1} in 2017.

Monitoring at Tigheci–Cociulia across the 2005–2025 calendar interval also provided 20 annual larval observations, with no larval assessment conducted in 2014. Mean *T. viridana* larval density was 3.39 ± 4.57 larvae 100 g^{-1} (CV = 134.7%), ranging from 0 in 2007 and 2008 to 17.57 larvae 100 g^{-1} in 2015. The maximum larval multiplication coefficient was 25.00 in 2018. Ecological egg-mass density was available for 20 years and averaged 5.57 ± 7.70 egg masses 100 g^{-1} (CV = 138.2%), reaching a maximum of 28.84 egg masses 100 g^{-1} in 2018. Viable egg-mass density per metre of branch was available for 16 years (2010–2025), averaging 1.86 ± 1.77 egg masses m^{-1} and reaching a maximum of 5.85 egg masses m^{-1} in 2018. In 2025, *T. viridana* larval density increased relative to 2024 at both sites, from 0.43 to 0.87 larvae 100 g^{-1} at Molești–Răzeni and from 1.95 to 10.31 larvae 100 g^{-1}

at Tigheci–Cociulia. Descriptive statistics for all population indicators are presented in Table 1.

Table 1. Descriptive statistics for *Tortrix viridana* egg-related indicators and for the larval densities and relative contributions of the three focal defoliator groups at the Molești–Răzeni and Tigheci–Cociulia monitoring sites during 2005–2025.

Variable	Site	<i>n</i>	Mean	SD	SE	Min	Max	Median	CV (%)
<i>T. viridana</i> ecological egg-mass density (egg masses 100 g ^{−1})	Molești–Răzeni	21	5.72	7.16	1.56	0.30	26.28	2.32	125.1
	Tigheci–Cociulia	20	5.57	7.70	1.72	0.40	28.84	3.17	138.2
<i>T. viridana</i> viable egg-mass density (egg masses m ^{−1})	Molești–Răzeni	11	1.53	1.88	0.57	0.11	5.85	0.76	122.9
	Tigheci–Cociulia	16	1.86	1.77	0.44	0.21	5.85	0.81	95.1
<i>T. viridana</i> larval density (larvae 100 g ^{−1})	Molești–Răzeni	20	2.89	4.30	0.96	0.21	18.04	1.08	149.0
	Tigheci–Cociulia	20	3.39	4.57	1.02	0.00	17.57	1.46	134.7
<i>T. viridana</i> relative contribution (%)	Molești–Răzeni	20	16.91	12.27	2.74	2.86	49.61	13.35	72.6
	Tigheci–Cociulia	20	14.40	14.16	3.17	0.00	46.65	11.18	98.3
Other Tortricidae larval density (larvae 100 g ^{−1})	Molești–Răzeni	20	17.08	35.27	7.89	0.66	154.55	4.38	206.5
	Tigheci–Cociulia	20	18.99	41.96	9.38	1.23	194.24	6.42	221.0
Other Tortricidae relative contribution (%)	Molești–Răzeni	20	57.98	23.86	5.34	22.32	94.43	54.72	41.2
	Tigheci–Cociulia	20	53.63	21.42	4.79	18.47	90.78	52.61	39.9
Pyralidae larval density (larvae 100 g ^{−1})	Molești–Răzeni	20	3.33	5.06	1.13	0.00	21.16	1.15	151.9
	Tigheci–Cociulia	20	4.53	2.71	0.61	1.29	11.74	3.90	59.8
Pyralidae relative contribution (%)	Molești–Răzeni	20	25.12	22.53	5.04	0.00	73.22	23.43	89.7
	Tigheci–Cociulia	20	31.97	21.88	4.89	4.55	76.10	25.37	68.4
Summed larval density of the three focal groups (larvae 100 g ^{−1})	Molești–Răzeni	20	23.30	38.64	8.64	0.87	173.30	10.52	165.8
	Tigheci–Cociulia	20	26.91	45.37	10.15	3.82	214.43	17.41	168.6

Notes: SD, standard deviation; SE, standard error; CV, coefficient of variation. Missing observations were excluded and were not treated as zero densities. Larval data were unavailable for Molești–Răzeni in 2015 and for Tigheci–Cociulia in 2014. Relative contribution was calculated within the assemblage formed by the three focal groups (*T. viridana*, other Tortricidae, and Pyralidae) and does not represent the complete oak-defoliator community. Ecological egg-mass density and viable egg-mass density per metre of branch are distinct indicators and were analysed separately.

Annual variation in *T. viridana* larval density at both monitoring sites during 2005–2025 is shown in Figure 2.

3.2. Relative Composition and Dynamics of the Three Focal Defoliator Groups

Within the three-group assemblage, *T. viridana* had the lowest mean relative contribution at both monitoring sites. Its mean relative contribution was $16.91 \pm 12.27\%$ at Molești–Răzeni and $14.40 \pm 14.16\%$ at Tigheci–Cociulia. Other Tortricidae had the highest mean relative contribution at both sites, accounting for $57.98 \pm 23.86\%$ of the three-group assemblage at Molești–Răzeni and $53.63 \pm 21.42\%$ at Tigheci–Cociulia. Pyralidae contributed a mean of $25.12 \pm 22.53\%$ at Molești–Răzeni and $31.97 \pm 21.88\%$ at Tigheci–Cociulia.

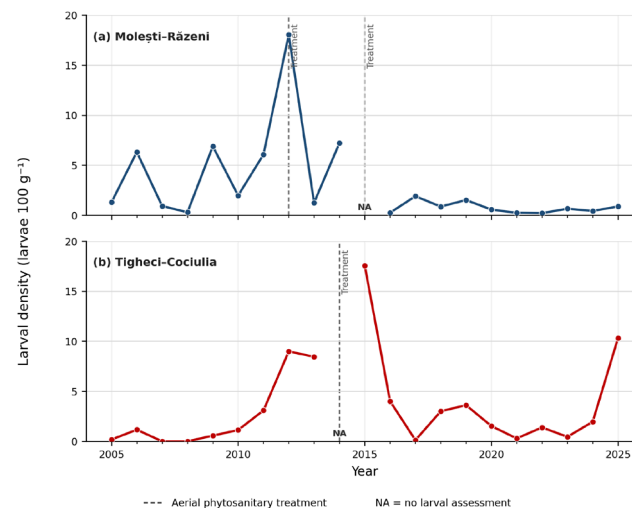


Figure 2. Annual variation in *Tortrix viridana* larval density at the Molești–Răzeni and Tigheci–Cociulia monitoring sites during 2005–2025. The blue line represents Molești–Răzeni, and the red line represents Tigheci–Cociulia. Vertical grey dashed lines indicate years in which aerial phytosanitary treatments were applied: 2012 and 2015 at Molești–Răzeni and 2014 at Tigheci–Cociulia. Breaks in the lines and the label “NA” indicate years without larval assessments (2015 at Molești–Răzeni and 2014 at Tigheci–Cociulia); missing observations were not treated as zero and were not interpolated. Larval density is expressed as larvae per 100 g of estimated fresh foliage mass.

The highest annual densities of other Tortricidae were recorded at Tigheci–Cociulia in 2013 (194.24 larvae 100 g^{−1}) and at Molești–Răzeni in 2012 (154.55 larvae 100 g^{−1}). These values exceeded the corresponding *T. viridana* densities of 8.45 and 18.04 larvae 100 g^{−1}, respectively. Annual changes in the relative composition of the three focal groups at both monitoring sites are shown in Figure 3.

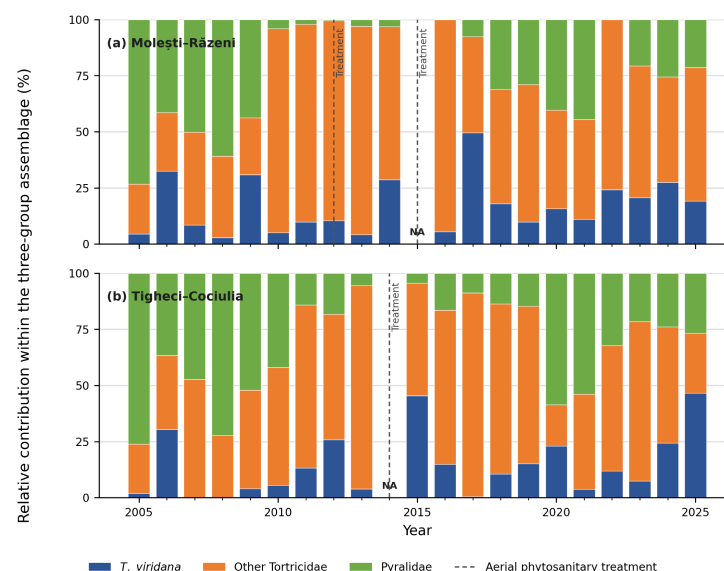


Figure 3. Annual relative composition of the three focal oak-defoliator groups at the (a) Molești–Răzeni and (b) Tigheci–Cociulia monitoring sites during 2005–2025. Relative contributions were calculated from the summed larval density of *Tortrix viridana*, other Tortricidae, and Pyralidae and therefore do not represent the complete oak-defoliator community. Dashed vertical lines indicate aerial phytosanitary treatments. Larval observations were unavailable for Molești–Răzeni in 2015 and for Tigheci–Cociulia in 2014 (NA).

3.3. Temporal Trends in Population Indicators

Results of the temporal trend analyses for the absolute larval densities of the three focal defoliator groups are summarized in Table 2. At Molești-Răzeni, significant decreasing trends were detected for *T. viridana* (Kendall's $\tau = -0.358$, adjusted $p = 0.045$) and Pyralidae ($\tau = -0.508$, adjusted $p = 0.045$). Other Tortricidae also showed a negative temporal tendency ($\tau = -0.442$), but this trend did not remain statistically significant after correction for temporal autocorrelation and multiple testing (adjusted $p = 0.057$).

Table 2. Temporal trends in the absolute larval densities of the three focal oak-defoliator groups at the Molești-Răzeni and Tigheci-Cociulia monitoring sites during 2005–2025.

Defoliator Group	Site	<i>n</i>	Test	Kendall's τ	Raw <i>p</i>	Adjusted <i>p</i>	Theil–Sen Slope (95% CI)
<i>T. viridana</i>	Molești-Răzeni	20	Standard MK	−0.358	0.030	0.045	−0.097 (−0.338 to −0.005)
Other Tortricidae	Molești-Răzeni	20	Modified MK	−0.442	0.057	0.057	−0.305 (−1.137 to −0.167)
Pyralidae	Molești-Răzeni	20	Modified MK	−0.508	0.028	0.045	−0.264 (−0.546 to −0.071)
<i>T. viridana</i>	Tigheci-Cociulia	20	Standard MK	0.216	0.194	0.545	0.084 (−0.060 to 0.302)
Other Tortricidae	Tigheci-Cociulia	20	Modified MK	0.105	0.653	0.653	0.142 (−0.500 to 1.050)
Pyralidae	Tigheci-Cociulia	20	Standard MK	−0.153	0.363	0.545	−0.102 (−0.320 to 0.116)

MK, Mann–Kendall test; Modified MK, Hamed–Rao variance-corrected Mann–Kendall test; CI, confidence interval. Adjusted *p*-values were calculated separately for each site using the Benjamini–Hochberg procedure across the three focal defoliator groups. Theil–Sen slopes represent annual changes in larval density (larvae 100 g^{−1} estimated fresh foliage year^{−1}). Statistical significance was evaluated using adjusted $p < 0.05$.

At Tigheci-Cociulia, none of the three focal groups showed a statistically significant temporal trend. The direction of change was positive for *T. viridana* ($\tau = 0.216$, adjusted $p = 0.545$) and Other Tortricidae ($\tau = 0.105$, adjusted $p = 0.653$), and negative for Pyralidae ($\tau = -0.153$, adjusted $p = 0.545$).

The estimated rates of decline at Molești-Răzeni were −0.097 larvae 100 g^{−1} year^{−1} for *T. viridana* and −0.264 larvae 100 g^{−1} year^{−1} for Pyralidae. Other Tortricidae exhibited a comparatively larger negative Theil–Sen slope (−0.305 larvae 100 g^{−1} year^{−1}), although the corresponding modified Mann–Kendall result did not meet the adjusted significance threshold. The apparent difference between the slope confidence interval and the modified Mann–Kendall significance result reflects the temporal-autocorrelation correction applied to the latter and should therefore be interpreted cautiously.

At Tigheci-Cociulia, all estimated slopes were relatively small and their confidence intervals included zero for *T. viridana* and Pyralidae. Consequently, the final data provide no statistical evidence of a consistent monotonic increase or decrease in the larval density of any focal group at this site. Sensitivity analyses excluding the 2012 treatment-year observation from the Molești-Răzeni series preserved the direction of the estimated trends, indicating that the observed patterns were not determined solely by that observation.

3.4. Relationships Between Defoliator Groups

Pairwise Spearman rank correlations showed a strong positive association between *T. viridana* and Other Tortricidae larval densities at Molești-Răzeni ($r_s = 0.779$, adjusted $p < 0.001$; Table 3). At Tigheci-Cociulia, the association between these groups was moderately positive ($r_s = 0.525$), but it did not remain statistically significant after correction for multiple testing (raw $p = 0.017$; adjusted $p = 0.052$). No significant associations involving Pyralidae were detected at either monitoring site (Table 3).

The positive association between *T. viridana* and Other Tortricidae at Molești-Răzeni remained strong when the 2012 treatment-year observation was excluded ($r_s = 0.742$, $p < 0.001$), indicating that the relationship was not determined solely by the unusually high densities recorded in that year. Nevertheless, these associations should not be interpreted

as evidence of direct ecological interactions or population synchrony, because both groups may respond similarly to shared environmental conditions, host phenology, monitoring conditions, or long-term temporal change. The absence of significant correlations involving Pyralidae suggests that annual variation in this group was not consistently associated with fluctuations in either *T. viridana* or Other Tortricidae.

Table 3. Pairwise Spearman rank correlations among the absolute larval densities of the three focal oak-defoliator groups at the Molești-Răzeni and Tigheci-Cociulia monitoring sites during 2005–2025.

Comparison	Site	<i>n</i>	Spearman's r_s	Raw <i>p</i>	Adjusted <i>p</i>
<i>T. viridana</i> vs. Other Tortricidae	Molești-Răzeni	20	0.779	<0.001	<0.001
<i>T. viridana</i> vs. Pyralidae	Molești-Răzeni	20	0.326	0.161	0.161
Other Tortricidae vs. Pyralidae	Molești-Răzeni	20	0.352	0.128	0.161
<i>T. viridana</i> vs. Other Tortricidae	Tigheci-Cociulia	20	0.525	0.017	0.052
<i>T. viridana</i> vs. Pyralidae	Tigheci-Cociulia	20	0.089	0.710	0.710
Other Tortricidae vs. Pyralidae	Tigheci-Cociulia	20	0.151	0.525	0.710

Adjusted *p*-values were calculated separately for each monitoring site using the Benjamini–Hochberg procedure across the three pairwise comparisons. Statistical significance was evaluated using adjusted *p* < 0.05.

3.5. Comparison Between Monitoring Sites

Paired Wilcoxon signed-rank tests conducted for the 19 years with observations available at both sites showed no statistically significant between-site difference in the larval density of any of the three focal defoliator groups after correction for multiple testing (Table 4).

Table 4. Paired comparisons of the absolute larval densities of the three focal oak-defoliator groups between the Molești-Răzeni and Tigheci-Cociulia monitoring sites during the 19 common monitoring years (2005–2013 and 2016–2025).

Defoliator Group	Molești-Răzeni Median (IQR)	Tigheci-Cociulia Median (IQR)	W	Raw <i>p</i>	Adjusted <i>p</i>	Rank-Biserial Correlation
<i>T. viridana</i>	0.92 (0.50–1.94)	1.39 (0.37–3.36)	93	0.952	0.952	−0.021
Other Tortricidae	4.24 (1.74–7.99)	6.30 (2.99–17.82)	78	0.507	0.760	−0.179
Pyralidae	1.32 (0.68–4.98)	3.90 (2.85–6.14)	57	0.131	0.394	−0.400

Larval densities are expressed as larvae 100 g^{−1} estimated fresh foliage. IQR, interquartile range; W, paired Wilcoxon signed-rank statistic. Negative rank-biserial correlations indicate higher ranks at Tigheci-Cociulia. Adjusted *p*-values were calculated using the Benjamini–Hochberg procedure across the three comparisons. Statistical significance was evaluated using adjusted *p* < 0.05.

The between-site difference was negligible for *T. viridana* (rank-biserial correlation = −0.021). Other Tortricidae also showed a relatively small between-site effect (−0.179). Pyralidae density was generally higher at Tigheci-Cociulia, with a median of 3.90 larvae 100 g^{−1} compared with 1.32 larvae 100 g^{−1} at Molești-Răzeni and a moderate rank-biserial effect of −0.400. However, this difference was not statistically significant after correction for multiple testing (adjusted *p* = 0.394). Therefore, the analysis did not provide statistical evidence of consistent differences between the two sites, although the moderate effect observed for Pyralidae may warrant further investigation.

4. Discussion

Long-term monitoring of *T. viridana* and two associated oak-defoliator groups in southern Moldova revealed pronounced interannual variability, with coefficients of variation exceeding 130% for *T. viridana* larval density at both sites. This degree of variability is consistent with the characteristic cyclic dynamics reported for *T. viridana* across its European

range, where prolonged periods of low abundance alternate with episodic outbreaks [27,28]. The highest *T. viridana* larval densities were recorded in 2012 at Molești-Răzeni and in 2015 at Tigheci-Cociulia. Although larval density increased at both sites between 2024 and 2025, the increase was substantially greater at Tigheci-Cociulia. Consequently, the available observations do not provide sufficient evidence to infer a common outbreak cycle or regional synchronization between the two sites. Interpretation of these fluctuations is further constrained by aerial treatments and missing larval assessments in different years at the two sites.

Long-term studies conducted in other forest systems demonstrate that Lepidoptera populations may combine pronounced interannual variability with longer-term changes in abundance and community composition. For example, a 15-year analysis of the oak processionary moth, *Thaumetopoea processionea* L., at three sites in Hungary identified strong synchronisation among populations and showed that aridity during May–July explained a substantial proportion of the annual variation in moth catches [40]. Multi-decadal observations of foliage-feeding Lepidoptera in Ukrainian deciduous forests have likewise documented changes in the abundance and relative importance of individual taxa, including *T. viridana* [16,17,28]. Long-term forest-health assessments from Romania and Serbia have similarly shown that defoliation patterns need to be interpreted in relation to interacting climatic and biotic stressors [41]. These findings indicate that long-term trends in oak-associated Lepidoptera must be interpreted against substantial interannual variability and in relation to weather, host-tree condition, and local forest characteristics.

Stand development may also contribute to differences among oak-associated insect communities. The monitored stands at Molești-Răzeni were 60–80 years old, whereas those at Tigheci-Cociulia were generally older, reaching 90–120 years. Studies conducted in oak forests have shown that stand age and associated structural characteristics can influence herbivore abundance, species richness, and community composition through changes in canopy complexity, microclimate, host-plant architecture, and resource availability [42]. However, the present study included only two forest sites, and stand age was not replicated independently of site. Therefore, the observed differences cannot be attributed directly to stand age. Moreover, previous research on the vitality structure and natural regeneration of *Q. robur* populations has emphasised the substantial variation among forest communities and the need for systematic monitoring of oak-stand condition [43]. Future monitoring should include replicated *Quercus robur* stands distributed across defined age classes and should record canopy structure, stand density, tree health, and microclimatic conditions together with defoliator abundance.

According to the records of the administering forestry enterprises, aerial spring treatments targeting the oak-defoliating Lepidoptera complex were applied at Molești-Răzeni in 2012 and 2015 and at Tigheci-Cociulia in 2014; information on the products and application parameters was unavailable, and the timing of the 2014 larval assessment relative to treatment could not be established. At Molești-Răzeni, lower larval densities were recorded after both treatment years, whereas at Tigheci-Cociulia the absence of a larval assessment in 2014 prevents evaluation of the immediate treatment response. Consequently, the observed population changes cannot be attributed to treatment because untreated control plots and sufficiently detailed treatment records were unavailable. An important finding of the present study is that *T. viridana* was not, on average, the dominant component among the three focal defoliator groups at either monitoring site. Other Tortricidae accounted for an average of 58.0% of the three-group larval density at Molești-Răzeni and 53.6% at Tigheci-Cociulia, whereas *T. viridana* accounted for 16.9% and 14.4%, respectively. These results indicate that monitoring focused exclusively on *T. viridana* may not adequately represent changes in the broader early-season tortricid assemblage. Comparable changes

in the composition of phytophagous Lepidoptera assemblages have been reported in Ukrainian deciduous forests, including shifts in the relative abundance of individual taxa and groups [16,17]. *T. viridana* and Other Tortricidae densities were strongly associated at Moleşti-Răzeni ($r_s = 0.779$, adjusted $p < 0.001$), whereas the moderate positive association at Tigheci-Cociulia did not remain significant after correction for multiple testing ($r_s = 0.525$, adjusted $p = 0.052$). Shared responses to oak phenology and early-season conditions are biologically plausible [18,19,21], but the present data do not identify the environmental mechanisms underlying these associations.

The predominance of Other Tortricidae within the three focal groups supports the continued recording of this group alongside *T. viridana* in phytosanitary monitoring. However, the present data are insufficient to support revision of intervention thresholds because taxa within the broad operational groups were not consistently resolved and actual defoliation was not quantified. After correction for temporal autocorrelation and multiple testing, Pyralidae larval density showed a significant decreasing trend at Moleşti-Răzeni ($\tau = -0.508$, adjusted $p = 0.045$; Theil–Sen slope = -0.264 larvae $100\text{ g}^{-1}\text{ year}^{-1}$), but not at Tigheci-Cociulia ($\tau = -0.153$, adjusted $p = 0.545$). No significant association between Pyralidae and either tortricid group was detected at either site. The results therefore do not support a regional decline in Pyralidae or a competitive relationship with Other Tortricidae. Because Pyralidae were recorded as a broad operational group, without consistent species-level resolution, the ecological mechanisms underlying the decline observed at Moleşti-Răzeni cannot be determined from the available data. The significant decrease in Pyralidae at Moleşti-Răzeni may also be considered within the broader context of long-term insect declines reported from Europe. Substantial reductions in insect biomass, abundance, and diversity have been documented in several long-term datasets [44], while studies focused specifically on moths indicate considerable heterogeneity among regions, habitats, ecological guilds, and individual species [45]. Forest insects do not respond uniformly: recent standardised monitoring across German forests detected declines in many species and sites, but responses differed among trophic and life-history groups, and herbivorous insects did not necessarily follow the same trends as other functional groups [46]. Therefore, the decline detected in the operational Pyralidae group at Moleşti-Răzeni should not be interpreted as direct evidence of a general regional insect decline, but it identifies a local pattern that warrants more taxonomically resolved investigation.

Only two aerial phytosanitary treatments were documented at Moleşti-Răzeni during the monitoring interval, in 2012 and 2015. These isolated interventions are unlikely to constitute the sole explanation for a declining trend extending across the complete time series. However, pesticide exposure cannot be excluded entirely because information was unavailable regarding the active substances, treated area, application rates, exposure from surrounding land uses, or other possible local interventions. Moreover, the Pyralidae records represent a broad operational group that may contain species with different host associations, phenologies, and responses to environmental change. More generally, assessments of operational management in Romanian public forests have identified substantial variability in technical planning and have emphasised the importance of standardised documentation and improved spatial recording of forest operations [47]. Future monitoring should therefore combine improved taxonomic identification, including rearing or molecular confirmation where feasible, with detailed records of forest management, pesticide exposure, stand structure, microclimate, and surrounding land use. Paired comparisons across the 19 common monitoring years detected no statistically significant difference between sites in the larval density of any focal group.

However, absence of statistical significance does not demonstrate equivalent defoliator pressure, particularly because Pyralidae showed a moderate between-site effect despite the

non-significant adjusted p -value. Given the high interannual variability and the inclusion of only two monitoring sites, the present study cannot distinguish regional drivers from effects associated with local stand conditions. Additional monitoring sites and explicit climatic and stand-level covariates would be required to evaluate these influences. *T. viridana* larval density increased between 2024 and 2025 at both sites, but remained low at Molești-Răzeni and reached 10.31 larvae 100 g⁻¹ at Tigheci-Cociulia. A single-year increase is insufficient to identify the onset of a new outbreak cycle; nevertheless, the increase observed at Tigheci-Cociulia supports continued monitoring, given the episodic population increases previously documented for this species [28]. Future research should prioritize the integration of climatic data, particularly spring temperature and precipitation, with population dynamics data across a larger network of monitoring sites in Moldova, using regression and time-series modeling approaches. Future monitoring should also improve the taxonomic resolution of the operational defoliator groups, record defoliation severity and complete treatment metadata, and, where possible, include untreated comparison plots.

5. Conclusions

Twenty annual larval assessments distributed across 2005–2025 were available for each monitoring site. The corrected series showed pronounced interannual variability in *T. viridana* and the two associated defoliator groups. After correction for temporal autocorrelation and multiple testing, significant decreases in *T. viridana* and Pyralidae larval densities were detected at Molești-Răzeni, whereas no significant temporal trend was detected for any focal group at Tigheci-Cociulia.

Other Tortricidae represented, on average, the largest component of the three-group assemblage, accounting for 58.0% at Molești-Răzeni and 53.6% at Tigheci-Cociulia, compared with 16.9% and 14.4%, respectively, for *T. viridana*. A strong positive association between *T. viridana* and Other Tortricidae was detected at Molești-Răzeni, but not at Tigheci-Cociulia after correction for multiple testing. No statistically supported between-site differences in larval density were identified across the 19 common monitoring years, although this should not be interpreted as evidence of equivalent defoliator pressure at the two sites.

These findings support monitoring *T. viridana* together with other consistently recorded oak-defoliator groups. However, broad operational taxonomic categories, missing assessments, incomplete treatment records, and the absence of direct defoliation measurements limit ecological and management interpretation. Continued monitoring should therefore improve taxonomic resolution, retain complete treatment metadata, quantify defoliation, and integrate climatic variables across a larger network of sites.

Author Contributions: Conceptualization, N.M., V.B., S.V., E.E. and G.N.; Methodology, V.B., N.M., S.V. and E.E.; Investigation, N.M., V.B., S.V., E.E. and V.I.; Formal analysis, N.M., V.B., G.N. and R.C.; Data curation, N.M., V.B., S.V., E.E., V.I. and G.N.; Visualization, R.C.; Writing—original draft preparation, R.C.; Writing—review and editing, N.M., R.C., V.B., G.N., S.V., E.E. and V.I.; Supervision, R.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was carried out within the Institutional Research Program, Subprogram No. 020407, “Development and Implementation of Good Practices for Sustainable Agriculture and Climate Resilience” (GREEN), funded by the Technical University of Moldova. The study was conducted as a joint scientific effort of the Technical University of Moldova and the Forest Research and Management Institute (ICAS Chișinău). Long-term field monitoring and data collection on forest defoliators were performed by specialists from ICAS Chișinău. The preparation and publication of this article received institutional support from the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Institutional Review Board Statement: Not applicable. This study did not involve human participants or vertebrate animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request. The long-term monitoring dataset on forest defoliators used in this study is archived at the Forest Research and Management Institute (ICAS Chişinău), Republic of Moldova.

Acknowledgments: The authors express their sincere gratitude to the staff of the Silvo-Hunting Enterprise “Sil-Răzeni” and the Forestry Enterprise “Silva-Sud Cahul” for their support in conducting field investigations and facilitating access to the study areas. During the preparation of this manuscript, the authors used Claude (Opus 5, Anthropic, San Francisco, CA, USA) for initial R code generation and data analysis, and ChatGPT (GPT-5.6, OpenAI, San Francisco, CA, USA) for data verification, R code revision, statistical workflow verification, and language editing. All outputs were independently reviewed and verified by the authors, who take full responsibility for the analyses and the content of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

CI	Confidence interval
CV	Coefficient of variation
FDR	False discovery rate
ICAS	Forest Research and Management Institute (Institutul de Cercetări şi Amenajări Silvice), Chişinău
IQR	Interquartile range
Kegg	Multiplication coefficient for egg-mass density
Klarv	Multiplication coefficient for larval density
MK	Mann–Kendall
SD	Standard deviation
SE	Standard error

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