# Evaluation of an AI-based Software System for Counting and Body Size Measurement of the Springtail *Folsomia candida* in OECD 232 Reproduction Tests

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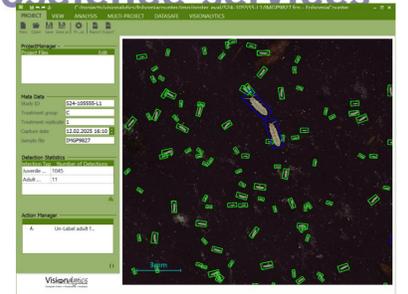
Background

The springtail reproduction test with *Folsomia candida* (OECD 232, 2016 [1]) is an essential part of the test battery for environmental risk assessment of plant protection products in the soil compartment. Even though the reproductive output for a valid requires 100 juveniles per control replicate at the end of the 28-day test period only, the actual number of juveniles is usually much higher and can reach values well over 1000. After testextraction from the test soil with dyed black water, the white coloured adult and juvenile springtails are floating on the water surface and can be counted manually either directly under the binocular or on the computer using photographs taken from the water surface. In either way, manual counting is very time consuming, laborious, and prone to error.

AI-based Counting Software

To facilitate, accelerate, and standardise the counting process, the software FOLSOMIACOUNTER was developed to enable automated adult and juvenile counting from photographs of the water surface after extraction. Additionally, an AI-based functionality allows estimating body size of the counted adult and juvenile springtails. This supports observers to look for sublethal effects other than reproduction.

Evaluation

Photographs from the yearly reference item studies of 2023 and 2024 with boric acid were analysed using the software with regard to (1) the achieved **time saving**, (2) the counting **accuracy**, (3) the **impact on the ecotoxicological endpoints** $EC_{10}/EC_{20}/EC_{50}$ and LC_{50} as well as (4) the potential of **body size measurement** (i.e., body length and body area) as an additional sublethal parameter. The number of adults and juveniles detected by the software was compared to the numbers after manual correction of false negatives/positives for determination of accuracy. Time saving was evaluated by comparison to manual counting without the use of the software. Conservative detection threshold (at precision: 99,6% and recall: 94,9%) was applied to minimize false positives, such that only missing animals have to be manually added.

Results & Conclusion

Time saving

The overall time saving was 59 % (per study: 1.6 hrs, per replicate: 4.2 min) for the 2023 study and 68 % (per study: 2.2 h, per replicate: 2.9 min) for the 2024 study. Time saving increased with the number of juveniles per replicate (**Fig. 1**).
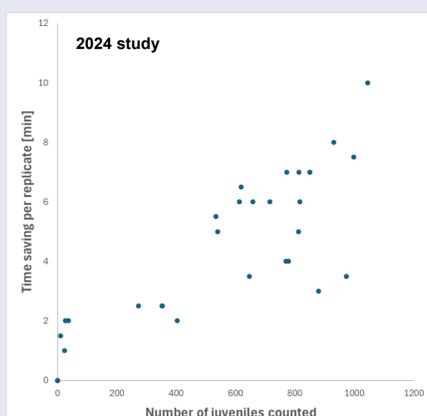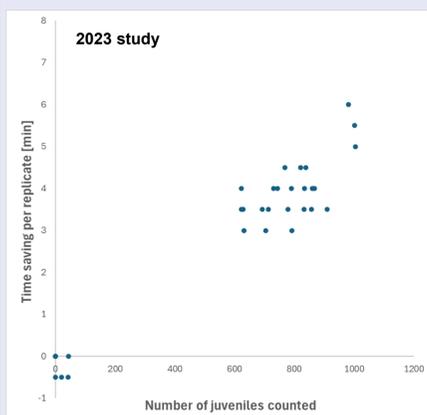

Fig. 1: Time saving per replicate in relation to the number of *Folsomia candida* juveniles present

Considerable time could be saved even if each counting was corrected for false positives and negatives by hand.

Accuracy

The overall accuracy was 86 % (2023 study) and 91 % (2024 study) for the adults as well as 97 % (2023 study) and 96 % (2024 study) for the juveniles. The slight discrepancies were mainly false positives when the number of juveniles was low and false negatives when the number of juveniles was high (**Fig. 2**).
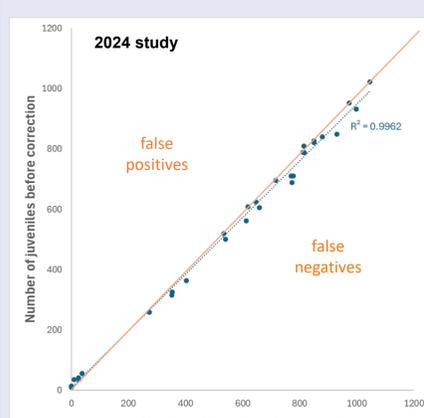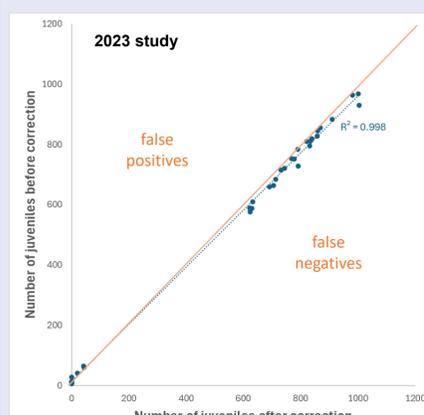

Fig. 2: Comparison of countings of *Folsomia candida* juveniles using the software without (before) and with (after) manual correction

Overall accuracy was greater than 95 % for the juveniles.

Impact on endpoints (LC_x/EC_x)

The endpoints based on the software countings alone were within the range of 1.66 and 1.87 (LC_{50}), 1.05 and 1.26 (EC_{10}), 1.02 and 1.15 (EC_{20}) and 0.98 and 1.02 (EC_{50}) when compared to the endpoints for the corrected countings (i.e., after elimination of false positives and addition of false negatives) (**Tab. 1** and **Tab. 2**).

Tab. 1: Comparison of endpoints for *Folsomia candida* before and after manual correction (values in brackets: 95 %-confidence interval) for the 2023 reference item study

Endpoints (2023 study)	Before manual correction	After manual correction
LC_{50}	99.4 (90.1 - 110)	186 (165 – 210)
EC_{10}	66.5 (46.8 – 78.7)	69.7 (36.2 – 84.6)
EC_{20}	76.3 (59.9 – 87.7)	78.2 (50.9 – 92.5)
EC_{50}	96.5 (84.1 – 109)	95.0 (80.0 – 110)

Tab. 2: Comparison of endpoints for *Folsomia candida* before and after manual correction (values in brackets: 95 %-confidence interval) for the 2024 reference item study

Endpoints (2024 study)	Before manual correction	After manual correction
LC_{50}	101 (91.6 – 111)	168 (149 – 190)
EC_{10}	39.9 (30.7 – 47.6)	50.3 (47.0 – 54.1)
EC_{20}	49.8 (40.8 – 57.9)	57.5 (54.1 – 61.4)
EC_{50}	72.7 (62.6 – 83.0)	74.2 (69.8 – 78.9)

The endpoints for the main parameter reproduction were not considerably influenced when using the software output solely without manual correction of false countings.

Body size measurement

Evaluation of body size measurement revealed an apparent concentration-dependent reduction in body length at the two highest concentrations (**Fig. 3**) and the existence of juveniles that hatched from two egg batches successively laid by the adults after intermediate moultings (**Fig. 4**).
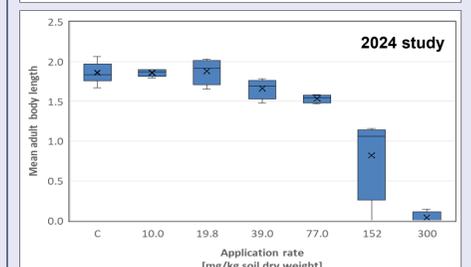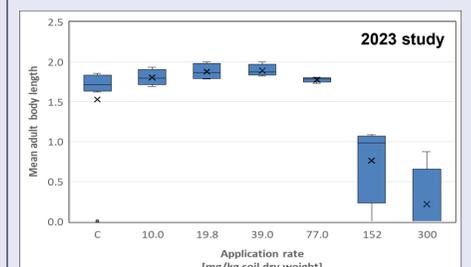

Fig. 3: Body length of adult *Folsomia candida* in relation to concentrations of boric acid in soil
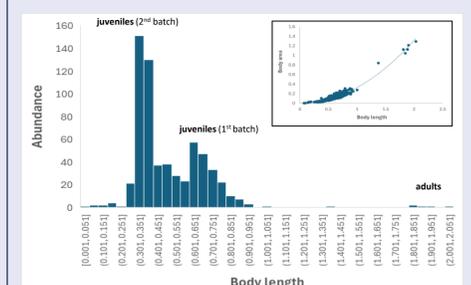

Fig. 4: Histogram of body length for a typical control replicate (inlay: relation between body size parameters body length and body area)

Body size measurement allows metric statistical evaluation of a second sublethal parameter besides reproduction.

References

[1] OECD (2016): Test No. 232: Collembolan Reproduction Test in Soil. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris.

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