

Food Authentication: The Detection of *Arbutus unedo* and *Olea europaea* Leaves as an Admixture of Oregano Using LAMP- and Duplex LAMP-Based Test Systems with Lateral-Flow Assays

Nathalie Holz, Nils Wax, Boris A. Illarionov, Margarita Iskhakova, Markus Fischer*

Hamburg School of Food Science, Institute of Food Chemistry, University of Hamburg,
Grindelallee 117, 20146 Hamburg, Germany

* Correspondence: markus.fischer@uni-hamburg.de; Tel. +49 40 42838-4359/7

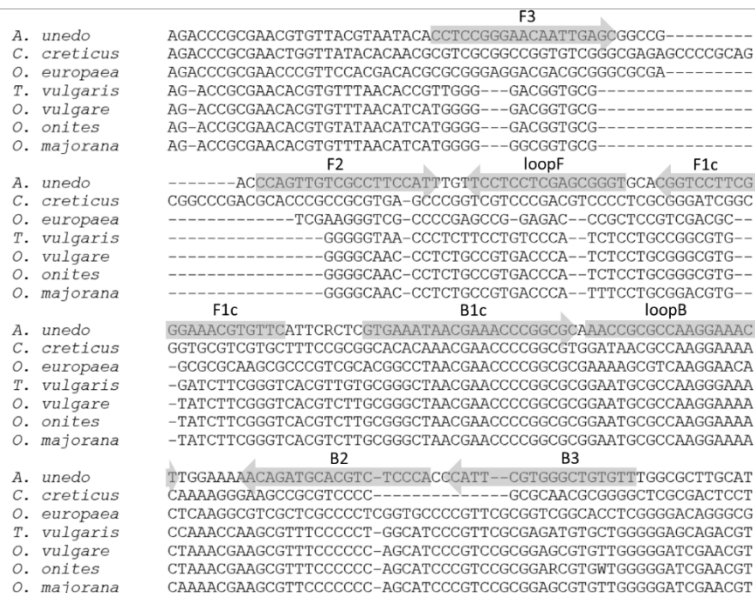


Figure S1. Multiple sequence alignment and phylogenetic tree of the internal transcribed spacer sequence of *A. unedo* (AF091952.1), *C. creticus* (MN579474.1), *O. europaea* (KU528507.1), *T. vulgaris* (AY329369.1), *O. vulgare* (DQ667243.1), *O. onites* (JX163054.1) and *O. majorana* (JX162957.1). Primer binding sites of the primer set specific for *A. unedo* are highlighted.

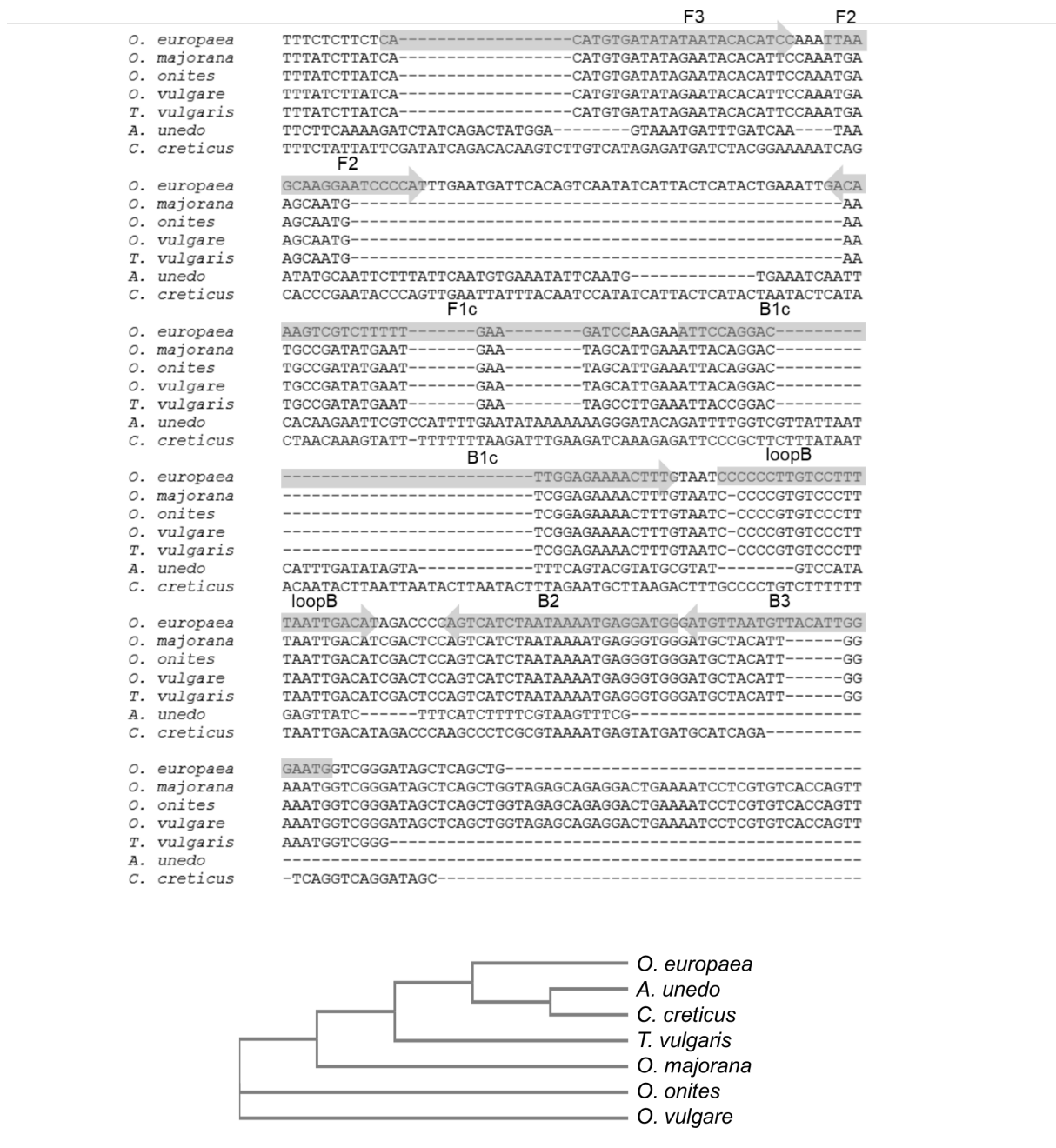
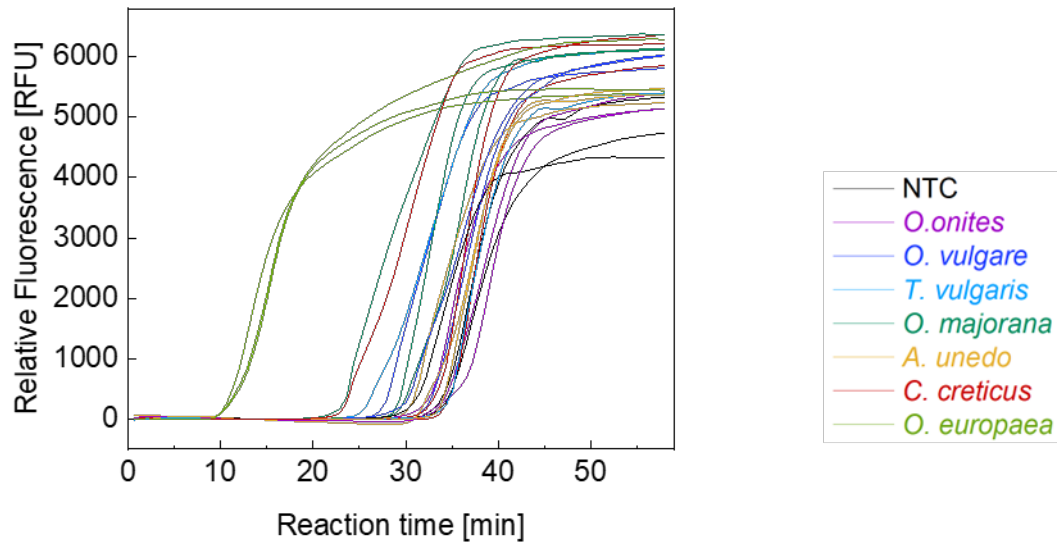


Figure S2. Multiple sequence alignment and phylogenetic tree of the trnL-trnF spacer sequence of *O. europaea* (MG255765.1), *O. majorana* (MT385088.1), *O. onites* (OL598301.1), *O. vulgare* (ON641383.1), *T. vulgaris* (GU381636.1), *A. unedo* (JQ067650.2) and *C. creticus* (MN579321.1). Primer binding sites of the primer set specific for *O. europaea* are highlighted.

a



b

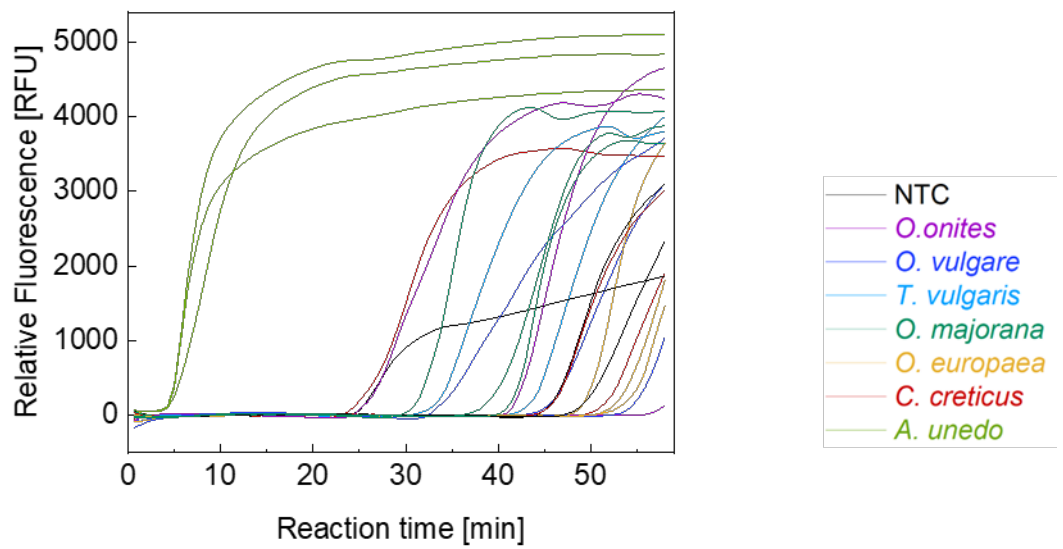


Figure S3. Real-time fluorescence LAMP with the final primerset specific for *O. europaea* (a) and *A. unedo* (b). Reaction conditions recommended by NEB for LAMP reactions were used in this experiment. The primer sets were tested for cross-reactivity with species listed in table 1 in the manuscript.

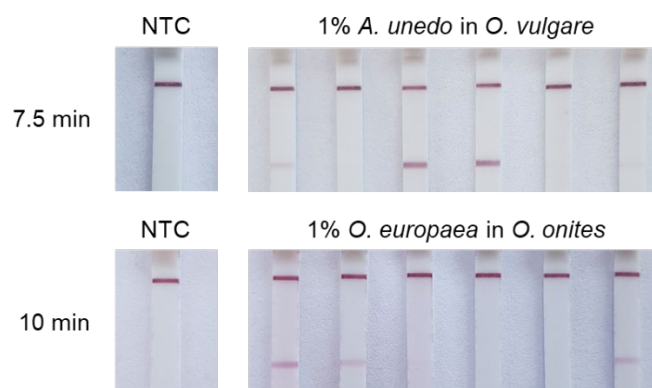


Figure S4. LAMP/LFA carried out with primer sets specific for olive tree (*O. europaea*) or strawberry tree (*A. unedo*) for admixture levels of 1% in oregano after 7.5 minutes or 10 minutes reaction time in 6-fold determination.

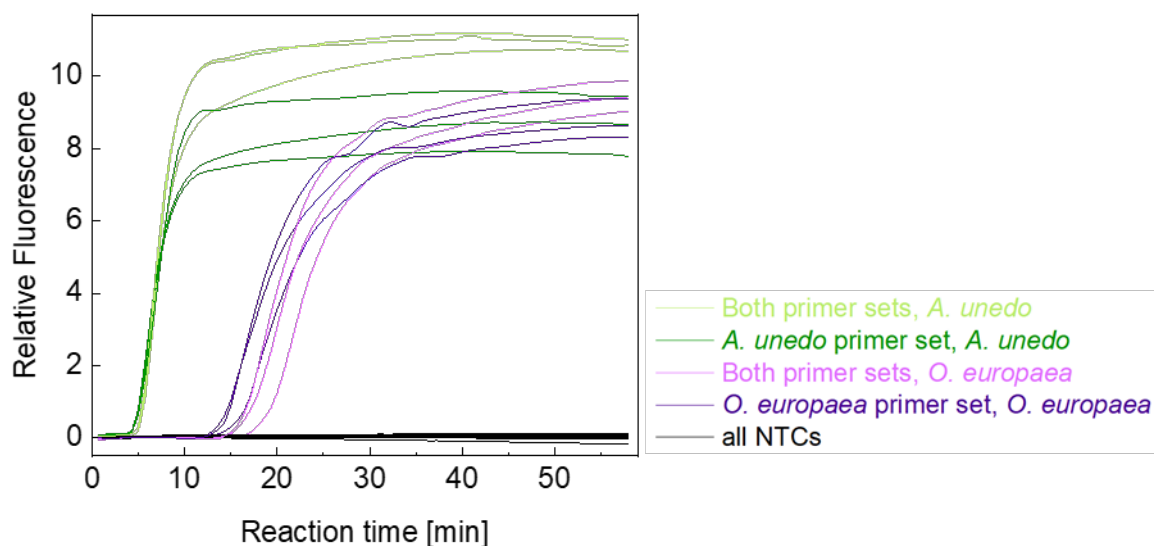


Figure S5. Real-time LAMP with primer sets specific for strawberry tree or olive tree with and without the addition of the respective other primer set. Light green: Both primer sets and *A. unedo* DNA sample; Dark green: *A. unedo* specific primer set and *A. unedo* DNA sample; Pink: Both primer sets and *O. europaea* DNA sample; Purple: *O. europaea* specific primer set and *O. europaea* DNA sample.

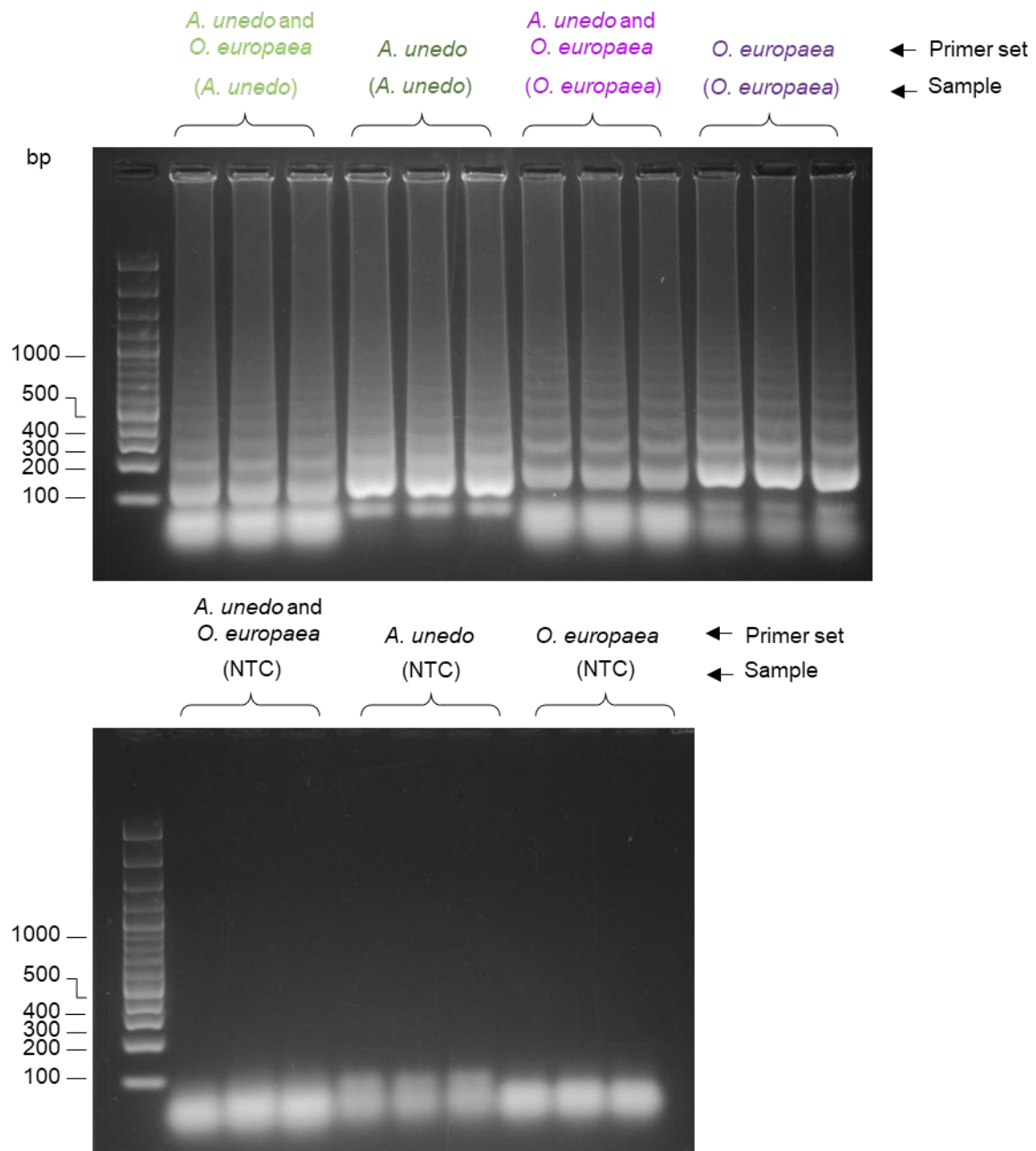


Figure S6. Agarose gel picture of the LAMP reaction products and NTC from Figure S5. Reactions with both sets of primers in one pot show more leftover primers (the band below the 100 bp mark) than the uniplex LAMP reactions, indicating the absence of amplification by the respective other primer set in the reaction.