



(51) International Patent Classification:

G01N 21/78 (2006.01)

G01N 1/22 (2006.01)

(21) International Application Number:

PCT/US2024/041756

(22) International Filing Date:

09 August 2024 (09.08.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/531,771

09 August 2023 (09.08.2023)

US

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(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: METHOD AND DEVICE FOR RAPID ONION VOC ANALYSIS

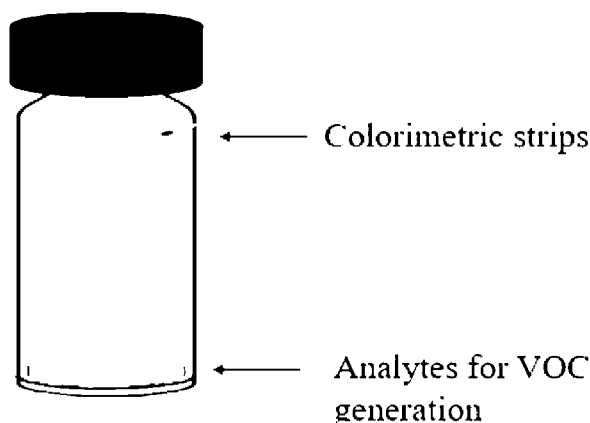


FIG. 1

(57) Abstract: Methods and systems for rapid detection of volatile organic compounds (VOCs) from plants, such as onions and other fruits and vegetables, are described. The systems include one or more colorimetric sensing elements and a cell-damaging component that can damage one or more cells in a plant sample to induce VOC release. In an exemplary system, a colorimetric dye array is combined with one or more needles on a single sensor body. Use of the system for classifying onion bulbs based on tearing intensity and/or pungency is described.

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

TITLE

METHOD AND DEVICE FOR RAPID ONION VOC ANALYSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to and benefit of U.S. Provisional Application Serial No. 63/531,771, filed on August 9, 2023, the disclosure of which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

10 The presently disclosed subject matter relates to methods and systems to detect volatile organic compounds (VOCs) from plants.

BACKGROUND

15 Many plants, including many plants used as foods or as food ingredients, contain and/or can emit volatile organic compounds (VOCs). The relative amounts and types of plant VOCs can vary depending upon a number of factors (e.g., species, hybrid, ripeness, sweetness, etc.). Thus, monitoring plant VOCs can provide useful information.

20 Accordingly, there is an ongoing need for methods and systems for monitoring plant VOCs, particularly in plant species of economic importance, such as those used as foods or as food ingredients. In particular, there is a need for rapid, inexpensive and/or minimally invasive methods and systems for monitoring plant VOCs, e.g., for determining fruit and vegetable quality. Further, there is a need to develop a practical technology for rapid detection
25 of volatiles in fruits and vegetables for using VOCs in breeding selection.

SUMMARY

30 This summary lists several embodiments of the presently disclosed subject matter, and in many cases lists variations and permutations of these embodiments. This summary is merely exemplary of the numerous and varied embodiments. Mention of one or more representative features of a given embodiment is likewise exemplary. Such an embodiment can typically exist with or without the feature(s) mentioned; likewise, those features can be

applied to other embodiments of the presently disclosed subject matter, whether listed in this summary or not. To avoid excessive repetition, this Summary does not list or suggest all possible combinations of such features.

5 In some embodiments, the presently disclosed subject matter provides a system for detecting one or more volatile organic compounds (VOCs) of interest in a plant sample, the system comprising: (a) one or more colorimetric sensing elements capable of undergoing a color change when contacted by one or more VOCs of interest or a chemical breakdown product thereof; and
10 (b) a cell-damaging component configured to damage at least one plant cell in the plant sample, thereby inducing release of one or more VOCs of interest from the plant sample. In some embodiments, the system further comprises a sensor body configured to hold (a) and/or (b) and/or to provide a semi-enclosed or enclosed cavity to receive VOC emissions released from the plant sample when the sensor body is contacted to a surface of the plant sample.

15 In some embodiments, the plant sample is a fruit, a vegetable, or a part thereof, and wherein the one or more VOCs of interest comprise one or more aldehyde, ketone, or sulfur-containing VOCs, optionally wherein the one or more VOCs of interest are associated with a species of fruit or vegetable and/or with a sensory property of the fruit or vegetable. In some embodiments,
20 the vegetable is an *Allium* plant, optionally *Allium cepa*, and the one or more VOCs of interest comprise one or more aldehyde, ketone, or sulfur-containing VOCs associated with a sensory property of an *Allium* plant, optionally wherein the sensory property is tearing intensity and/or pungency and/or wherein the one or more VOCs of interest include one or more of the group
25 consisting of propanethial S-oxide, 2-methyl-2-pentenal, 4-methyl-2-pentenal, 4-methyl-3-heptanone, and 3,4-dimethylthiophene.

In some embodiments, the one or more colorimetric sensing elements comprise one or more colorimetric nanosensors and/or one or more colorimetric dyes. In some embodiments, the one or more colorimetric
30 sensing elements comprise a plurality of colorimetric sensing elements, optionally at least 5, at least 10, or at least 15 colorimetric sensing elements. In some embodiments, the plurality of colorimetric sensing elements comprise a plurality of colorimetric dyes, optionally wherein each of the plurality of colorimetric dyes is selected from the group comprising a base indicator, an

acid indicator, a solvatochromic dye, an amine-based dye, and a metal salt, optionally wherein the amine-based dye comprises a porphyrin moiety.

5 In some embodiments, the plurality of colorimetric sensing elements are arranged in an array on a surface of a sensor substrate, optionally wherein the sensor substrate is paper or nitrocellulose. In some embodiments, the sensor substrate is configured on a first surface of a sensor body, optionally wherein the sensor body further comprises one or more additional surfaces extending perpendicularly from the first surface, wherein when the sensor body is contacted to a surface of a plant sample while or after the plant sample is contacted with the cell-damaging component, the sensor body forms an enclosed or semi-enclosed cavity to receive VOCs released from the plant sample and wherein said VOC can contact the colorimetric sensing elements of the sensor substrate.

15 In some embodiments, the cell-damaging component comprises one or more needles, optionally wherein each or the one or more needles comprises a metal, silica, glass, and/or polymer body having a base and a tip. In some embodiments, the one or more needles are configured to penetrate the plant sample to a depth of at least about 5 millimeters (mm), optionally to a depth of at least about 10 mm, at least about 15 mm, at least about 20 mm, 20 at least about 25 mm, at least about 30 mm, or at least about 35 mm.

In some embodiments, the sensor comprises a sensor body comprising a first surface and one or more additional surfaces extending perpendicularly from the first surface, wherein a sensor substrate comprising an array of a plurality of colorimetric sensing elements is configured on the first surface of the sensor body, and wherein the one or more needles are provided in or on 25 the sensor body and configured where the body of each needle extends perpendicularly from the first surface, wherein when the sensor body is contacted to a plant sample while the plant sample is contacted with the tip of the one or more needles, the sensor body forms enclosed or semi-enclosed cavity to receive VOCs released from the plant sample and where the VOCs 30 can contact the colorimetric sensing elements of the sensor substrate. In some embodiments, the system further comprises a detector for detecting a color change of the one or more colorimetric sensing elements, optionally wherein said detector comprises a camera, an optical microscope, or a

smartphone reader, further optionally wherein the system is provided as a handheld or other portable device.

5 In some embodiments, the presently disclosed subject matter provides a method of detecting one or more volatile organic compounds (VOCs) of interest in a plant, the method comprising: (i) contacting a plant sample with a cell-damaging component configured to damage at least one plant cell in the plant sample to induce release of one or more VOCs of interest from the plant sample; (ii) providing one or more colorimetric sensing elements, wherein one or more of the one or more colorimetric sensing agents undergo
10 a color change when one or more VOCs of interest are released by the contacting of (i); and (iii) detecting a color change associated with one or more of the one or more colorimetric sensing elements, thereby detecting one or more VOCs of interest. In some embodiments, the plant sample is a sample of a fruit or a vegetable, and wherein the one or more VOCs of interest
15 comprise one or more aldehyde, ketone, or sulfur-containing VOCs, optionally wherein the one or more VOCs of interest are associated with a species of fruit or vegetable and/or with a sensory property of the fruit or vegetable.

In some embodiments, the vegetable is an *Allium* plant, optionally *Allium cepa*, and wherein the one or more VOCs of interest comprise one or
20 more aldehyde, ketone, or sulfur-containing VOCs associated with a sensory property of an *Allium* plant. In some embodiments, the plant sample is a bulb or leaf of an *Allium* plant, optionally of an *Allium cepa* plant.

In some embodiments, the contacting comprises puncturing the surface of the plant sample, optionally wherein the puncturing is performed
25 with one or more needles and/or wherein the puncturing penetrates the plant sample to a depth of at least about 5 mm. In some embodiments, the one or more colorimetric sensing elements comprises a plurality of colorimetric sensing elements provided in an array.

In some embodiments, the detecting comprises analyzing a color
30 change profile in the array, wherein analyzing the color change profile comprises determining which of the plurality of colorimetric sensing elements in the array change color over a pre-determined time period and/or determining a temporal order of color change of at least two or more colorimetric sensing elements in the array. In some embodiments, the

analyzing further comprises comparing the color change profile in the array to a color change profile provided by performing the method on a control plant sample having one or more known properties. In some embodiments, the detecting is performed visually. In some embodiments, the detecting is performed using a camera, an optical microscope, or a smartphone reader. In some embodiments, the detecting provides information related to the tearing intensity and/or pungency of an *Allium* plant, optionally an *Allium cepa* plant. In some embodiments, the method is performed at a site where the plant is grown, stored, or sold.

In some embodiments, the presently disclosed subject matter provides a method of selecting an *Allium* plant having a desired tearing intensity and/or pungency, the method comprising: (i) damaging at least one cell of an *Allium* plant, wherein the damaging induces release of a plurality of volatile organic compounds (VOCs) from the damaged cell of the *Allium* plant but does not impact the viability of the *Allium* plant; (ii) contacting the plurality of VOCs with an array comprising a plurality of colorimetric sensing elements; (iii) detecting a color change profile in the array, wherein detecting the color change profile comprises determining which of the plurality of colorimetric sensing elements in the array change color over a pre-determined time period and/or determining a temporal order of color change of at least two or more colorimetric sensing elements in the array; and (iv) determining the tearing intensity and/or pungency of the *Allium* plant based on the color change profile of step (iii). In some embodiments, the *Allium* plant is selected from an onion plant, a shallot plant, a leek plant, and a garlic plant, optionally wherein the *Allium* plant is an onion plant.

In some embodiments, the damaging is performed by puncturing the *Allium* plant, optionally wherein the puncturing is performed using one or more needles; by cutting the *Allium* plant; by grinding the *Allium* plant, or by crushing a portion of the *Allium* plant. In some embodiments, the pre-determined time period is about 2 minutes. In some embodiments, step (iv) comprises comparing the color change profile from (iii) with a color change profile of one or more control *Allium* plants of known tearing intensity and/or pungency. In some embodiments, the method comprises selecting a tearless *Allium* plant.

In some embodiments, the presently disclosed subject matter provides a method of developing an *Allium* plant in an *Allium* plant breeding program, the method comprising: (i) providing a plurality of *Allium* plants; (ii) determining one or more sensory properties of each of the plurality of *Allium* plants based on color change data from one or more colorimetric sensing elements contacted with volatile organic compounds (VOCs) from the *Allium* plant, optionally wherein the color change data comprises a color change profile from an array comprising a plurality of colorimetric sensing elements and/or wherein the color change data is provided using a system or method disclosed herein for detecting one or more VOCs of interest; and (iii) using the one or more sensory properties determined in (ii) for crossing, selection, or advancement decisions in an *Allium* plant breeding program. In some embodiments, step (iii) comprises: (iii-a) selecting a first *Allium* plant having a first property of interest; (iii-b) selecting a second *Allium* plant having a second property of interest; and (iii-c) crossing said first *Allium* plant and said second *Allium* plant to provide a hybrid *Allium* plant; optionally wherein said method further comprises (iii-d) selecting progeny of said hybrid *Allium* plant having one or more properties of interest for further breeding. In some embodiments, the hybrid *Allium* plant is a tearless *Allium* plant.

In some embodiments, the presently disclosed subject matter provides a computer-implemented method of determining a tearing intensity and/or pungency of an *Allium* plant, the method comprising: (i) receiving data representing a tearing intensity and/or pungency of an *Allium* plant using one or more colorimetric sensing elements, optionally wherein the data comprises a color change profile of an array of colorimetric sensing elements contacted with volatile organic compounds (VOCs) from the *Allium* plant and/or wherein said data is provided by using a system or method disclosed herein for detecting one or more VOCs of interest; (ii) comparing the data from (i) to a database comprising data collected from colorimetric sensing elements contacted with VOCs from reference *Allium* plants, wherein the comparing is performed using a computer processor; and (iii) generating an output indicating the tearing intensity and/or pungency of the *Allium* plant based on (ii). In some embodiments, the *Allium* plant is an onion plant.

Accordingly, it is an object of the presently disclosed subject matter to provide systems and related methods to detect VOCs in a plant sample, for example, in an onion or other *Allium* species sample, as well as related methods of selecting, developing, or determining a tearing intensity and/or pungency of an *Allium* plant. This and other objects are achieved in whole or in part by the presently disclosed subject matter.

An object of the presently disclosed subject matter having been stated above, other objects and advantages of the presently disclosed subject matter will become apparent to those of ordinary skill in the art after a study of the following description of the presently disclosed subject matter.

BRIEF DESCRIPTION OF THE FIGURES

Reference will now be made to the accompanying drawings, which are not necessarily drawn to scale.

Figure 1 is a schematic drawing of a testing device for assessing the responsiveness of colorimetric dyes to exposure to pure volatile organic compound (VOC) samples. Commercially available pure VOC samples are placed in the bottom of a vial. A nitrocellulose acetate strip printed with colorimetric dyes is inserted at the top of the vial and the vial is closed. Color change of individual dye spots is analyzed visually and/or by recorder after 60 minutes.

Figure 2 is a pair of photographic images showing an approach for analyzing the open-air responsiveness of exemplary 4x4 arrays of colorimetric dyes to volatile organic compounds (VOCs) of chopped onion samples from four different onion genotypes of varying tearing intensity. Colorimetric dyes were printed on nitrocellulose acetate membranes in 4x4 arrays (lower photographic image). Onions were peeled, chopped, and placed in a small uncovered petri dish. Colorimetric dye arrays were placed in a larger covered petri dish with the chopped onion samples (upper photographic image). Arrays were removed and analyzed at four different exposure times: 15 minutes, 30 minutes, 60 minutes, and 16 hours.

Figure 3A is a composite color change map showing the colorimetric dye response of an exemplary 16 dye array strip to volatile organic compounds (VOCs) from a tearing onion genotype (referred to as genotype

“B” herein) using the open-air responsiveness approach shown in Figure 2. Results are in triplicate for strips showing dye responsiveness at (from top to bottom for each 4-row strip) 15 minutes, 30 minutes, 1 hour, and 16 hours.

Figure 3B is a composite color change map showing the colorimetric dye response of an exemplary 16 dye array strip to volatile organic compounds (VOCs) from a tearing onion genotype (referred to as genotype “A” herein) using the open-air responsiveness approach shown in Figure 2. Results are in triplicate for strips showing dye responsiveness at (from top to bottom for each 4-row strip) 15 minutes, 30 minutes, 1 hour, and 16 hours.

Figure 3C is a composite color change map showing the colorimetric dye response of an exemplary 16 dye array strip to volatile organic compounds (VOCs) from a tearless onion genotype (referred to as genotype “C” herein) using the open-air responsiveness approach shown in Figure 2. Results are in triplicate for strips showing dye responsiveness at (from top to bottom for each 4-row strip) 15 minutes, 30 minutes, 1 hour, and 16 hours.

Figure 3D is a composite color change map showing the colorimetric dye response of an exemplary 16 dye array strip to volatile organic compounds (VOCs) from a tearless onion genotype (referred to as genotype “D” herein) using the open-air responsiveness approach shown in Figure 2. Results are in triplicate for strips showing dye responsiveness at (from top to bottom for each 4-row strip) 15 minutes, 30 minutes, 1 hour, and 16 hours.

Figure 4 is a graph showing the time-related responsiveness of colorimetric dyes to volatile organic compounds (VOCs) from four different onion genotypes (designated herein “A”, “B”, “C”, and “D”) based on the results of the open-air sampling studies described in Figures 3A-3D. Temporal responsiveness is indicated by the order of dyes from top (earliest dyes to change color) to bottom (dyes that change color after longer exposure to cut onion samples).

Figure 5A is a schematic diagram of an exemplary sensor device of the presently disclosed subject matter. The device body is a cap with four needles inserted in holes on the top side of the cap, extending through the holes so that the needle tips extend from the same side of the body surface as the side to which a colorimetric dye array is attached. The tips of the needles extend past the side of the cap so that when the device is contacted to plant sample,

the needles can puncture the sample and the device body can form an enclosed space together with the outer surface of the plant sample.

Figure 5B is a photographic image of an exemplary sensor device of the presently disclosed subject matter in contact with an onion bulb. The device body is a cap with needles inserted in holes on the top side of the cap, extending through the holes and inside the cavity formed by the cap to contact and puncture the onion surface. A colorimetric dye array (not visible in the photographic image) is attached on the inside surface of the cap where it is exposed to volatile organic compounds (VOCs) released by puncture of the onion by the tips of the needles.

Figure 6 is a composite color change map showing colorimetric dye responsiveness to an onion using a volatile organic compound (VOC) sensing device as shown in Figures 5A and 5B. The top panel shows the color change maps from a tearing onion genotype in an exemplary 16 dye array after 2, 5, 15, or 30 minutes. The bottom panel compares the color change pattern map from a tearing onion genotype to the color change pattern map from a tearless onion genotype after 2 minutes.

Figure 7A is a composite color change map showing the color change patterns of an exemplary 16 dye array to 26 tearing onions. Color change was assessed in duplicate for two minutes for each of the bulbs. The bulbs included in the boxes were also assessed via a blind study using human sensory testing (test exposure time 10-15 seconds).

Figure 7B is a composite color change map showing the color change patterns of an exemplary 16 dye array to 26 tearless onions. Color change was assessed in duplicate for two minutes for each of the bulbs. The bulbs included in the boxes were also assessed via a blind study using human sensory testing (test exposure time 10-15 seconds).

Figure 8A is a composite color change map showing the color change patterns of an exemplary 16 dye array to 20 onions (onion samples 1-20) from a total sample group of 100 onions comprised of 40 onions of tearing genotypes and 60 onions of tearless genotypes. Each sample is marked with the letter N or T in parentheses, indicating that the onion sample is a Tearless or Tearing type of onion, respectively.

Figure 8B is a composite color change map showing the color change patterns of an exemplary 16 dye array to 20 onions (onion samples 21-40) from a total sample group of 100 onions comprised of 40 onions of tearing genotypes and 60 onions of tearless genotypes. Each sample is marked with the letter N or T in parentheses, indicating that the onion sample is a Tearless or Tearing type of onion, respectively.

Figure 8C is a composite color change map showing the color change patterns of an exemplary 16 dye array to 20 onions (onion samples 41-60) from a total sample group of 100 onions comprised of 40 onions of tearing genotypes and 60 onions of tearless genotypes. Each sample is marked with the letter N or T in parentheses, indicating that the onion sample is a Tearless or Tearing, respectively.

Figure 8D is a composite color change map showing the color change patterns of an exemplary 16 dye array to 20 onions (onion samples 61-80) from a total sample group of 100 onions comprised of 40 onions of tearing genotypes and 60 onions of tearless genotypes. Each sample is marked with the letter N or T in parentheses, indicating that the onion sample is a Tearless or Tearing type of onion, respectively.

Figure 8E is a composite color change map showing the color change patterns of an exemplary 16 dye array to 20 onions (onion samples 81-100) from a total sample group of 100 onions comprised of 40 onions of tearing genotypes and 60 onions of tearless genotypes. Each sample is marked with the letter N or T in parentheses, indicating that the onion is a Tearless or Tearing type of onion, respectively.

Figure 9 is a comparison of the volatile organic compound (VOC) profiles of tearless versus tearing onions by solid-phase microextraction gas chromatography/mass spectrometry (SPME-GC-MS) for thiopropanal S-oxide, where the individual tearless genotypes (designated herein as genotypes "E", "F", "G", "H", "I", and "J") are represented on the left side of the solid vertical line and the individual tearing genotypes (designated herein as genotypes "K", "L", "M", and "N") are represented to the right of the solid vertical line.

Figure 10 is a comparison of the volatile organic compound (VOC) profiles of tearless versus tearing onions by solid-phase microextraction gas

chromatography/mass spectrometry (SPME-GC-MS) for 2-methyl-2-pentenal, where the individual tearless genotypes (designated herein as genotypes “E”, “F”, “G”, “H”, “I”, and “J”) are represented on the left side of the solid vertical line and the individual tearing genotypes (designated herein as genotypes “K”, “L”, “M”, and “N”) are represented to the right of the solid vertical line.

Figure 11 is a comparison of the volatile organic compound (VOC) profiles of tearless versus tearing onions by solid-phase microextraction gas chromatography/mass spectrometry (SPME-GC-MS) for 4-methyl-3-heptanone, where the individual tearless genotypes (designated herein as genotypes “E”, “F”, “G”, “H”, “I”, and “J”) are represented on the left side of the solid vertical line and the individual tearing genotypes (designated herein as genotypes “K”, “L”, “M”, and “N”) are represented to the right of the solid vertical line.

Figure 12 is a comparison of the volatile organic compound (VOC) profiles of tearless versus tearing onions by solid-phase microextraction gas chromatography/mass spectrometry (SPME-GC-MS) for 3,4-dimethylthiophene, where the individual tearless genotypes (designated herein as genotypes “E”, “F”, “G”, “H”, “I”, and “J”) are represented on the left side of the solid vertical line and the individual tearing genotypes (designated herein as genotypes “K”, “L”, “M”, and “N”) are represented to the right of the solid vertical line.

DETAILED DESCRIPTION

Many plants contain and/or can be induced to emit volatile compounds. Monitoring these volatile organic compounds (VOCs) can be useful in assessing the quality and/or shelf life of a variety of vegetables and fruit, such as, but not limited to, onion, melon (e.g., cantaloupe, honeydew), watermelon, berries, apples, tomato, carrot, and broccoli. The VOCs can remain metabolically active after harvest. Thus, produce quality assessment can be performed based on the emission of various VOCs that are produced by the action of enzymes, such as peroxidases, naturally present in the produce and which act on substrates also present in the produce. These VOCs can be associated with freshness, flavor or another quality appealing to consumers.

Often, the enzymes are present in different cellular compartments than the corresponding substrate. Thus, production of VOCs can be aided by cellular disruption to mix the enzymes and substrates. Storage conditions and other produce treatment conditions can affect enzyme activity and alter expected VOC emissions.

In fruit, VOCs are essential for fruit flavor, and along with sugars and acids, play a key role in the perception and acceptability of fruits by consumers. In melons for example, the most abundant volatile compounds are commonly alcohols, esters, and aldehydes. Fan et al. (*Front. Plant Sci.*, 16 March 2021, Sec. Plant Metabolism and Chemodiversity, Volume 12 – 2021) identified 20 volatiles that enhance sweetness independently of sugars, such as benzaldehyde, (*E*)-2-pentenal, nonanal, mesifurane, ethyl butanoate, and ethyl hexanoate. In tomatoes, alcohols, aldehydes, and ketones have been shown to be the prominent VOCs. See Zhang, Jing, et al., *Plants* (Basel). 2023 Aug; 12(16): 2947.

Plants of the genus *Allium* emit commonly encountered volatile compounds. Different types of onions are used worldwide as a common food or food ingredient. Some onion genotypes (tearing) emit reactive sulfur compounds after the surface is punctured, causing individuals in close proximity to the onion to produce tears. Other onion genotypes (tearless), produce the same volatiles, but in much lower quantities.

More particularly, within intact onion cells, the enzyme alliinase is compartmentalized in the cell vacuole and the flavor precursors are found in the cytoplasm. A reaction occurs when onion cells are damaged, and the enzyme and substrate are brought together as organelles are disrupted. The kinetics of decomposition are different for each specific flavor precursor. The decomposition of 1-propenyl cysteine sulfoxide is almost instantaneous, while the methyl and propyl cysteine sulfoxide decomposition occur in several minutes. Primary products produced from flavor precursor decomposition include pyruvate, ammonia, and chemically unstable sulfenic acids. Among the sulfenic acids is the lachrymator or tear-producing compound (i.e., thiopropanal sulfoxide, also known as thiopropanal S-oxide) characteristic of onions. The sulfenic acids undergo further rearrangement to form thiosulfinates, which are responsible for the characteristic flavor of onions.

The current standard method for plant VOC quality assessment, including onion quality assessment, involves gas chromatography-mass spectrometry (GC-MS), which is less than ideal, as GC-MS is not suitable for *in-situ*, rapid VOC analysis and the different sulfur compounds emitted by onions can degrade quickly. Additionally, onion VOCs are typically induced by mechanical cutting. A less invasive and rapid onion sampling method would be more advantageous.

In some embodiments, the presently disclosed subject matter provides methods, devices and systems to detect VOCs related to tearing intensity and/or pungency in Allium plants and VOCs related to other commercial attributes in fruit, vegetable, and root plants. The presently disclosed systems can include single platform devices that include a colorimetric sensor assay and a cell-damaging component for inducing VOC release from a plant sample, thus combining sampling and detection of VOCs in a single, portable, minimally invasive device that can be used for rapid and simultaneous sampling and VOC analysis.

Thus, provided in accordance with some embodiments of the presently disclosed subject matter is a system for VOC sensing of plant samples (e.g., vegetable or fruit samples). In some embodiments, the system comprises one or more colorimetric sensing elements and a cell-damaging component. The cell damaging component can comprise, for example, one or more components that can puncture or cut the skin or rind of the plant sample, such as, but not limited to, needles, punches, tacks, nails, picks, corers, or blades. In some embodiments, the components of the VOC sensor can serve two different purposes: introducing minimum mechanical damage to the sample to release volatiles, and sensing elements to detect VOCs *in situ* by color change. The combination of these two technologies can streamline conventional fruit and vegetable VOC analysis, while not relying only on more subjective methods, such as sensory perception.

By providing for the simultaneous detection of VOCs, the presently disclosed and claimed subject matter is particularly suited to detection of VOCs that require cell damage for release (e.g., by allowing enzymes from one cellular compartment to contact substrates from another cellular compartment and/or by providing for a more concentrated release of VOCs

than would occur without cellular damage), as well as for VOCs that degrade over relatively short time periods following release (e.g., minutes or hours). For instance, the presently disclosed subject matter is useful for detection of VOCs related to tearing and/or pungency intensity in *Allium* plants, such as in onions. Thus, in some embodiments, the presently disclosed subject matter provides methods for selecting an *Allium* plant having a desired tearing and/or pungency intensity, for developing an *Allium* plant in an *Allium* plant breeding program, and in computer-implemented methods of determining the tearing and/or pungency intensity of an *Allium* plant. Moreover, the presently disclosed systems and methods are amenable to use in the field, for example, in locations where plants are grown, stored, sold and/or consumed.

The presently disclosed subject matter will now be described more fully hereinafter with reference to representative embodiments. The presently disclosed subject matter can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the embodiments to those skilled in the art.

I. DEFINITIONS

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently claimed subject matter.

Following long-standing patent law convention, the terms "a", "an", and "the" refer to "one or more" when used herein, including in the claims.

As used herein, the term "about", when referring to a value or an amount, for example, relative to another measure, is meant to encompass variations of in some embodiments $\pm 20\%$, in some embodiments $\pm 10\%$, in some embodiments $\pm 5\%$, in some embodiments $\pm 1\%$, and in some

embodiments $\pm 0.1\%$ from the specified value or amount, as such variations are appropriate. The term “about” can be applied to all values set forth herein.

As used herein, ranges can be expressed as from “about” one particular value, and/or to “about” another particular value. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as “about” that particular value in addition to the value itself. For example, if the value “10” is disclosed, then “about 10” is also disclosed. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

As used herein, the term “and/or” when used in the context of a listing of entities, refers to the entities being present singly or in combination. Thus, for example, the phrase “A, B, C, and/or D” includes A, B, C, and D individually, but also includes any and all combinations and sub-combinations of A, B, C, and D.

The term “comprising”, which is synonymous with “including,” “containing,” or “characterized by” is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. “Comprising” is a term of art used in claim language which means that the named elements are present, but other elements can be added and still form a construct or method within the scope of the claim.

As used herein, the phrase “consisting of” excludes any element, step, or ingredient not specified in the claim. When the phrase “consists of” appears in a clause of the body of a claim, rather than immediately following the preamble, it limits only the element set forth in that clause; other elements are not excluded from the claim as a whole.

As used herein, the phrase “consisting essentially of” limits the scope of a claim to the specified materials or steps, plus those that do not materially affect the basic and novel characteristic(s) of the claimed subject matter.

With respect to the terms “comprising”, “consisting of”, and “consisting essentially of”, where one of these three terms is used herein, the presently disclosed and claimed subject matter can include the use of either of the other two terms.

As used herein, “significance” or “significant” relates to a statistical analysis of the probability that there is a non-random association between two or more entities. To determine whether or not a relationship is “significant” or has “significance”, statistical manipulations of the data can be performed to calculate a probability, expressed in some embodiments as a “p-value”. Those p-values that fall below a user-defined cutoff point are regarded as significant. In some embodiments, a p-value less than or equal to 0.05, in some embodiments less than 0.01, in some embodiments less than 0.005, and in some embodiments less than 0.001, are regarded as significant.

The term “detecting” as used herein can refer to determining the presence or absence of a named material. In some embodiments, determining the presence of a named material can further comprises determining the amount or concentration or a relative amount or concentration of the named material. Thus, “detecting” can refer to simply detecting the presence or absence of a compound or group of compounds, as well as to quantitative or semi-quantitative detection.

The term “sensory property” as used herein refers to a property of a plant that is associated with a sensation perceived by a person. In some embodiments, the sensory property relates to appearance or another property detectable by the eyes (e.g., tearing), to smell, taste, and/or touch.

“Tearing” as used herein means any irritation upon exposure to a product (i.e., a volatile produced by a plant) that can be sensed with the eyes (i.e., produces tears). In some embodiments, tearing intensity can be measured on a scale from 1 to 7, where 1 indicates no irritation/no noticeable irritation (a score of 1 would indicate a “True Tearless” onion genotype, for example) and 7 indicates strong irritation/very noticeable irritation. A score of 1 to 3, for marketing/commercial purposes, can indicate a Tearless plant or plant genotype (e.g., for an *Allium* plant, such as an onion). Thus, in some embodiments, the term “Tearless” can be used for both “True Tearless” plants (i.e., an onion or genotype with a score of 1) and plants with relatively low/less tearing (i.e., an onion or genotype with a score of 2 or 3).

“Pungent” or “pungency” as used herein refers to a heat or burning intensity/sensation of a product upon chewing and/or consumption. The

heating or burning intensity/sensation can often be felt in a person's mouth, throat, nose, and/or chest area.

As used herein, "onion plant" or "onion" is a plant of the botanical species *Allium cepa* or parts thereof, such as the bulb (e.g., the harvested bulb), seeds, etc.

By "bulb" or "onion bulb" is meant the commercially harvested, edible portion of the onion plant. An onion bulb is comprised of concentric, enlarged fleshy leaf bases, also called scales. Onion bulbs can be developing onion bulbs or mature onion bulbs. In some embodiments, the onion bulb is a mature onion bulb.

II. SYSTEMS AND METHODS FOR DETECTING PLANT VOCs

As described hereinabove, there remains an ongoing need for rapid, practical methodology and inexpensive systems for determining fruit and vegetable quality. In many cases, desirable properties can be linked to VOCs emitted by the fruits and vegetables or that can be triggered to be released from the fruits and vegetables via damaging one or more cells or cell components of the fruit or vegetable. These VOCs can be related to particular sensory characteristics. Fruit and vegetable VOCs related to sensory characteristics of interest include, but are not limited to, VOCs associated with flavor characteristics (e.g., esters (melon); aldehydes and ketones (watermelon); terpenes (carrot); sulfur-volatiles (onion), etc); pungency and irritation (e.g., 2-methyl-2-pentenal (onion)); and signs of ripening stage (e.g., 2-methyl-butyl-acetate (melon)).

For example, as described above, the tearing intensity and pungency of onions is related to VOCs produced when onion cells are damaged. Current technology for determining onion tearing intensity is/can be subjective, for example, judged on a scale of 1-7 based on sensory perception of irritation; or, alternatively, can involve expensive and time-consuming techniques, such as gas chromatography/mass spectrometry (GC-MS). GC-MS can include time- and labor-intensive sample preparation, e.g., liquid extraction or solid-phase microextraction (SPME); relatively long analysis time (e.g., 45 minutes per sample) and low throughput; and the use of expensive, non-portable equipment.

Additional desirable produce properties, such as sweetness/low pungency and other flavor qualities also lack practical assessment approaches. For example, current methods for assessing onion sweetness include pyruvate testing, where the designation for a sweet onion is a pyruvate score of less than 5 $\mu\text{mol/g}$. However, this method is not reliable, creating imposter sweet onion brands on the market. Yet, as with onion tearing intensity, assessment of onion sweetness via SPME-GC-MS is labor- and time-intensive and is typically performed at the research stage only. Thus, there remains a need for a rapid and practical tool to confirm onion sweetness by volatile analysis at the commercial level. The flavor qualities of other produce, particularly fruits (such as melons, berries, watermelon, and tomato), can include the use of refractometry to measure total soluble solid (%Brix) or sugars. A VOC sensor for fruits could also be a useful tool in providing non-subjective assessment of compounds associated with desirable flavor profiles in these crops, either alone or in combination with refractometry.

As described hereinbelow, an exemplary colorimetric sensing system was developed for detecting VOCs related to tearing intensity and pungency in onions (e.g. onion bulbs). First, a group of colorimetric sensing elements sensitive to different chemical functional groups present in onion VOCs was assessed and a set of VOCs was selected to provide an exemplary sixteen-member sensor array. The time-dependent response of the colorimetric sensor array to VOCs from cut tearing and tearless onions was studied. In one evaluation, four different onion genotypes (having between them four different tearing intensity levels, as confirmed by sensory perception assessment and GC-MS methods) were tested using an exemplary sensor system of the presently disclosed subject matter that includes a bottle cap with the sensor array attached on the inside and one or four needles placed in holes in a bottle cap. See Figures 5A and 5B. In a second evaluation, ten different onion genotypes (having different tearing intensity levels, as confirmed by sensory perception assessment and GC-MS methods) were tested using an exemplary colorimetric sensor system of the presently disclosed subject matter that include a bottle cap with the sensor array attached on the inside and one or four needles placed in holes in a bottle cap. After an exposure time of two minutes to the punctured onions, differences in

color change pattern in the sensor array were suitable for categorizing the onion genotypes based on tearing intensity. In addition to SPME-GC-MS, other methods for independently assessing VOCs for comparison to the sensor system results include solid-phase microextraction, electronic-nose, and proton transfer reaction mass spectrometry, which are well-known in the art.

While the exemplary colorimetric sensing system was used to detect VOCs in onions, the system can also be adapted for use with any type of fruit or vegetable emitting a VOC, including but not limited to, melons (cantaloupe, honeydew), watermelon, tomato, and vegetables including but not limited to, carrots.

Accordingly, in some embodiments, the presently disclosed subject matter provides a system for detecting one or more VOCs of interest in a plant sample. The presently disclosed system comprises a colorimetric sensing element for detecting a VOC of interest and a component for inducing (e.g., triggering and/or enhancing) release of a VOC from the sample. Thus, the component for inducing VOC release can result in initiation of VOC release and/or provide a higher amount or concentration of VOC release. In some embodiments, the system comprises: (a) one or more colorimetric sensing elements capable of undergoing a color change when contacted by one or more VOCs of interest or a chemical breakdown product thereof; and (b) a cell-damaging component configured to damage at least one plant cell in the plant sample, thereby inducing release of one or more VOCs of interest from the plant sample.

In some embodiments, the system further comprises a body (i.e., a "sensor body") configured to hold or support (a, i.e., the one or more colorimetric sensing elements) and/or (b, i.e., the cell-damaging component). In some embodiments, the sensor body can receive and hold VOC emissions from a plant and/or direct them to the one or more colorimetric sensing elements. For example, in some embodiments, the sensor body can provide a semi-enclosed or enclosed cavity to receive VOC emissions released from the plant sample when the cell-damaging component and/or the sensor body is contacted to a surface of the plant sample. In some embodiments, the system includes a single sensor body that is configured to hold or position (a)

and (b) so that (b) can damage one or more plant cells in the plant sample while simultaneously bringing (a) into position to contact VOCs released from the plant sample. Thus, the system can be used to detect VOCs that degrade or react rapidly.

5 In some embodiments, the plant sample comprises a fruit, a vegetable, or a part thereof. In some embodiments, the part is an edible part of the fruit or vegetable (e.g., a root, flower, fruit, leaf, stem, bulb, pod, tuber, or rhizome). In some embodiments, the plant sample comprises a plant or a part thereof that produces VOCs of interest after cell damage (e.g., to mix enzymes from
10 one cellular compartment with a substrate or substrates in another cellular compartment). In some embodiments, the plant sample comprises a skin that impedes release of VOCs from the plant sample. In some embodiments, the plant is of the genus *Allium* (e.g., an onion plant sample, a shallot plant sample, a leek plant sample, or a garlic plant sample). In some embodiments,
15 the plant is an onion plant (*Allium cepa*). In some embodiments, the plant sample is an onion bulb. In some embodiments, the plant sample is selected from the group comprising melons such as but limited to cantaloupe, and honeydew, and other fruits such as watermelon and tomatoes, and vegetables, such as carrot. In some embodiments, the plant sample is
20 selected from the group including, but not limited to, apple, apricot, artichoke, asparagus, avocado, banana, beans, beet, blackberry, blueberry, *Brassica*, broccoli, Brussels sprouts, cabbage, carrot, cassava, cauliflower, celery, cherry, eggplant, endive, figs, grape, grapefruit, ground cherry, kiwifruit, lettuce, lemon, lime, mango, mushroom, okra, orange, papaya, pea, peach,
25 pepper, persimmon, pineapple, plantain, plum, pomegranate, potato, pumpkin, radicchio, radish, raspberry, spinach, strawberry, sugar beet, sugarcane, sweet potato, tangerine, and zucchini.

 VOCs of interest can be categorized by the chemical functional group or groups they contain. For example, VOCs can include functional groups
30 including, but not limited to aldehydes, ketones, alcohols, esters, and sulfur-containing groups (e.g., sulfides, disulfides, trisulfides, tetrasulfides, thiols, thiolanes, sulfoxides, and thiophenes). Different colorimetric sensing elements can be used depending upon the chemical functional group or groups with which they interact. In some embodiments, the one or more VOCs

of interest comprise one or more aldehyde, ketone, or sulfur-containing functional group (i.e., are aldehyde, ketone or sulfur-containing VOCs). VOCs of interest can be those associated with a particular type (e.g., a particular species) or types of vegetable or fruit. In some embodiments, VOCs of interest for fruit or vegetable samples can be those associated with a sensory property such as, but not limited to, a flavor profile (e.g., a sweetness level), a ripeness level, tearing intensity or pungency. Thus, in some embodiments, the presently disclosed system can be used to detect one or more VOCs as a proxy for flavor quality. In some embodiments, the sensory property can be affected by storage conditions (e.g., temperature, light level, or time).

In some embodiments, the plant is an *Allium* plant, optionally *Allium cepa*, and the one or more VOCs of interest comprise one or more aldehyde, ketone, or sulfur-containing VOCs associated with a sensory property of an *Allium* plant. In some embodiments, the sensory property relates to a flavor, tearing intensity, or pungency profile. In some embodiments the sensory property relates to tearing intensity and/or pungency and/or the one or more VOCs of interest include one or more of the group including, but not limited to, propanethial S-oxide (thiopropional sulfoxide), 2-methyl-2-pentenal, 4-methyl-2-pentenal, 4-methyl-3-heptanone, and 3,4-dimethylthiophene. In some embodiments, the sensory property is tearing intensity and/or the one or more VOCs of interest include one or more of the group including, but not limited to, propanethial S-oxide (thiopropional sulfoxide), 2-methyl-2-pentenal, 4-methyl-2-pentenal, 4-methyl-3-heptanone, dimethyl sulfide, and 3,4-dimethylthiophene. In some embodiments, the VOCs of interest include one or more of propanethial S-oxide, 2-methyl-2-pentenal, 4-methyl-2-pentenal, 4-methyl-3-heptanone, and 3,4-dimethylthiophene. In some embodiments, the sensory property is pungency and/or the one or more VOCs of interest include one or more of the group including, but not limited to, 2-methyl-2-pentenal, 4-methyl-2-pentenal, 4-methyl-3-heptanone, and 3,4-dimethylthiophene. Additional VOCs of interest for *Allium* plants include, but are not limited, to, decanal, 2,3-dimethyl-2,5-dihydrothiophene-2-one, 2,4-dimethyl-3-hexanol, 3-mercapto-2-methylpropanol, 1-[2-(isobutyryloxy)-1-methylethyl]-2,2-dimethylpropyl-2-methylpropanoate, 2-hydroxymethylcyclopropane-carboxylic acid methyl ester, trans-2-hydroxycyclohexanyl acetate, 1,5-

hexadien-3-ol acetate, dipropyl disulfide, *trans*-propenyl propyl disulfide, *cis*-di-1-propenyl disulfide, 1-propyl-2-(thiohept-2-en-5-yl) disulfide, diallyl disulfide, methyl-*trans*-propenyl disulfide, methyl-*cis*-propenyl disulfide, dipropyl trisulfide, *cis*-propenyl propyl trisulfide, di-1-propenyl trisulfide, *trans*-propenyl propyl trisulfide, dimethyl trisulfide, methyl propyl trisulfide, *cis*-methyl propenyl trisulfide, *trans*-methyl propenyl trisulfide, di-1-propenyl tetrasulfide, dimethyltetrasulfide, n-decyl sulfide, 2-(methylthio)propane, dithio(1-propenyl)propionate, hexyl vinyl sulfide, *trans*-Zwiebelane, *cis*-Zwiebelane, allyl thiopropionate, *cis*-2-ethyl-3-methylthiophene, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, *trans*-2-ethyl-3-methylthio-phenene, 2-acetylthiophene, 2,3-dimethyl-2,5-dihydrothiophene-2-one, 2-methyl-5-(methylsulfanyl)thiophene, 2-mercapto-3,4-dimethyl-2,3-dihydrothiophene, 3-mercapto-2-methylpropanol, isobutyl isothiocyanate, dipropyl sulfoxide, 4,6-diethyl-1,2,3,5-tetrathiolane, 5,7-diethyl-1,2,3,4,6-pentathiolane, 1,2-dithiolane, 1,2-dithiolane-1,1-dioxide, 4-methyl-3H-1,2-dithiol-3-thione, 3,5-diethyl-1,2,4-trithiolane, 4,6-diethyl-1,2,3,5-tetrathiolane, 2-vinyl-1,3-dithiane, 3-vinyl-1,2-dithiacyclohex-5-ene, hexathiepane, and 4-methyl-3-H-1,2-dithiol-3-one.

Any suitable colorimetric sensing element can be used. For instance, suitable colorimetric sensing elements include colorimetric nanosensors and colorimetric dyes. Thus, in some embodiments, the one or more sensing elements comprise a nanosensor, a dye, or a combination thereof. In some embodiments, fluorescent nanomaterials/dyes can be used to provide a fluorescent VOC sensor array. Accordingly, in some embodiments, nanomaterials and/or dyes can be used to provide a sensor that provides a colorimetric and/or fluorescent signal when the sensing element(s) are contacted with a VOC of interest.

Colorimetric and/or fluorescent nanosensors and dyes are known in the art. In some embodiments, the colorimetric nanosensor can take advantage of localized surface plasmon resonance (LSPR), which can result in wavelength specific adsorption of the nanosensor, e.g., in gold or silver nanoparticle-based nanosensors. Changes in the environment around the nanosensors, such as aggregation caused by interaction with a VOC, can

result in a change in wavelength specific adsorption that can be perceived as a color change.

In some embodiments, the colorimetric nanosensor for use in a system of the presently disclosed subject matter comprises a nanoparticle, a nanorod, and/or other shapes. Thus, in some embodiments, the nanosensor is “shape-controlled.” By way of additional example and not limitation, other shapes include cubes, prisms, discs, and the like. In some embodiments, the nanosensor has a dimension ranging from about 5 nanometers (nm) to about 200 nm, including 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 175, 180, 185, 190, 195, or 200 nm. In some embodiments, the nanosensor has an aspect ratio ranging from about 1 to about 6, including aspect ratios of 1, 2, 3, 4, 5, or 6, and fractional values therebetween, e.g. 2.5, 3.4, and the like. By way of particular example and not limitation, the nanosensor comprises a nanoparticle that has a dimension ranging from about 5 to about 200 nanometers (nm). In some embodiments, the nanosensor comprises a nanorod that has an aspect ratio of about 1 to about 6.

In some embodiments the nanosensor comprises a material selected from the group comprising gold, silver, copper, aluminum, or an alloy thereof. In some embodiments, the nanosensor comprises a nanoparticle and/or nanorod that comprises gold. In some embodiments, the nanosensor is functionalized with one or more ligand that reacts with the one or more VOC of interest emitted by the plant sample after the cell-damaging component damages one or more plant cells in the plant sample. For example, in some embodiments, the ligand can interact with a VOC functional group, such as but not limited to, an aldehyde, ketone or sulfur-containing functional group.

In some embodiments, the colorimetric sensing agent comprises one or more colorimetric dyes. The one or more colorimetric dyes can be selected from the group including, but not limited to, base indicators, acid indicators, solvatochromic dyes, metal salts, and amine-based dyes (e.g., amine-based dyes that comprise a porphyrin or metal-porphyrin complex). For instance, the one or more colorimetric dyes can comprise a plurality of cross-reactive dyes to probe a wide range of chemical properties of a single analyte (i.e., a single VOC) or a mixture of analytes (i.e., a mixture of VOCs). The chemical

interactions can include Lewis and Brønsted acidity/basicity, molecular polarity, redox property, and solvatochromism associated with plant vapor emissions.

5 In some embodiments, the system comprises a plurality of colorimetric sensing elements (e.g., to detect a plurality of VOCs from the same sample), such as a plurality of colorimetric nanosensors, a plurality of colorimetric dyes, or a combination of colorimetric nanosensors and colorimetric dyes. In some
10 embodiments, the use of a plurality of colorimetric sensing elements can provide semi-quantitative information regarding the VOCs of interest, e.g., if two or more sensing elements have different sensitivities to the same VOC or the same VOC-related functional groups). In some embodiments, the one or more colorimetric sensing elements comprise at least 2-24 colorimetric sensing elements (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 colorimetric sensing elements). In some
15 embodiments, the system can include more than 24 colorimetric sensing elements. In some embodiments, the one or more colorimetric sensing elements comprise at least 4 colorimetric sensing elements (e.g., at least 4 colorimetric dyes). In some embodiments, the one or more colorimetric sensing elements comprise at least 5 colorimetric sensing elements (e.g., at
20 least 5 colorimetric dyes). In some embodiments, the one or more colorimetric sensing elements comprise at least 10 colorimetric sensing elements (e.g., at least 10 colorimetric dyes). In some embodiments, the one or more colorimetric sensing elements comprise at least 15 colorimetric sensing elements (e.g., at least 15 colorimetric dyes). In some embodiments, the
25 colorimetric sensing elements comprise 16 colorimetric sensing elements (e.g., 16 colorimetric dyes).

In some embodiments, the colorimetric sensing elements are arranged in an array format. For example, each of the colorimetric sensing elements can be printed individually in an array on a surface of a sensor substrate. Any
30 suitable sensor substrate can be used. In some embodiments, the sensor substrate is a material that does not itself release a VOC that causes a color change in one or more of the colorimetric sensing elements and/or does not provide for diffusion of the colorimetric sensing elements under conditions present during assessment of plant sample VOCs. In some embodiments,

the sensor substrate comprises paper or nitrocellulose (e.g., a nitrocellulose acetate membrane).

Alternatively, in some embodiments, the plurality of colorimetric sensing elements can be integrated into the cell-damaging component. For instance, when the cell-damaging component comprises one or more needles, the colorimetric sensing elements can be configured in a pattern on a coating of one or more needle. In some embodiments, the cell-damaging component can comprise a plurality of needles arranged in an array format and each needle can be coated with a coating comprising a different colorimetric sensing element. In some embodiments, the needle bodies can be formed from a gas-permeable polymeric matrix and the one or more colorimetric sensing elements can be incorporated into the polymeric matrix of the needle bodies. Thus, in some embodiments, the cell-damaging component can comprise a needle array (e.g., a microneedle array patch) that also acts as the sensor substrate/colorimetric sensor array.

In some embodiments, the cell-damaging (e.g., puncturing) component comprises one or more needles, tacks, or hole punches. In some embodiments, the cell-damaging component comprises one or more needles. Each needle, for example, can comprise a body with a base end and a tip end. In some embodiments, the body can be tapered, such that the diameter of the tip end is smaller than the diameter of the base end. In some embodiments, the body is not tapered and the diameter of the tip end and the base end is the same. The needle body can be solid or hollow, porous or smooth. The needle body can comprise any material that can provide a needle having a hardness sufficient to damage one or more plant cells in the plant sample and/or to penetrate any plant skin or other outer covering of the plant sample. In embodiments, the one or more needles have a metal (e.g., stainless steel), silica, glass, ceramic, and/or polymer body. In some embodiments, the one or more needles can be provided as microneedles (e.g., as a polymeric microneedle array).

Any desired length of needle body can be employed. The length of the needle body can depend on the desired penetration depth of the plant sample. The desired penetration depth can depend on the type of plant sample. For example, for onion bulb samples, in some embodiments, it can be desirable

to penetrate not only the outer skin of the onion bulb or first scale of the bulb, but also to penetrate to the second, third, fourth, or fifth layer of onion scales. In some embodiments, the one or more needles are configured to penetrate the plant sample to a depth of at least about 1.5 millimeters (mm), optionally
5 at least about 3.0 mm, at least about 5.0 mm, at least about 10 mm, at least about 15 mm, at least about 20 mm, at least about 25 mm, at least about 30 mm, at least about 35 mm, at least about 40 mm, at least about 45 mm, or at least about 50 mm. In some embodiments, the one or more needles are configured to penetrate the plant sample to a depth of about 5 mm to about
10 35 mm. In some embodiments, the one or more needles are configured to penetrate the plant sample to a depth of about 10 mm to about 35 mm. In some embodiments, the one or more needles are configured to penetrate the plant sample to a depth of about 1 cm to 2.5 cm.

In some embodiments, e.g., for fruits with rinds, such as melons, the
15 fruit can be cut (e.g., with a knife or other bladed instrument) and the VOCs (e.g., the VOCs related to flavor quality) emitted from the flesh detected via a colorimetric dye array. In some embodiments, at least a portion of the flesh of the cut fruit can be placed in an enclosed (e.g., airtight) container with the colorimetric dye array for a period of time.

In some embodiments, the system comprises a sensor body (e.g., a
20 plastic, glass, or metallic sensor body) on which the sensor substrate (e.g., an array of colorimetric sensing elements on a paper or nitrocellulose substrate) is configured. In some embodiments, the sensor substrate is configured on a first part or surface of a sensor body and the sensor body can further
25 comprises one or more additional parts or surfaces extending perpendicularly from the first surface, wherein when the sensor body is contacted to a surface of a plant sample while or after the plant sample is contacted with the cell-damaging component, the sensor body forms an enclosed or semi-enclosed cavity to receive VOCs released from the plant sample and where the VOCs
30 released from the plant sample can come into contact with the colorimetric sensing elements of the sensor substrate. In some embodiments, the cell-damaging component is also incorporated into the sensor body. For example, in some embodiments, the sensor comprises a sensor body comprising a first surface and one or more additional surfaces extending perpendicularly from

the first surface, wherein a sensor substrate comprising an array of a plurality of colorimetric sensing elements is configured on the first surface of the sensor body, and wherein the one or more needles are provided in or on the sensor body and configured where the body of each needle extends perpendicularly from the first surface, wherein when the sensor body is contacted to a plant sample while the plant sample is contacted with the tip of the one or more needles, the sensor body forms enclosed or semi-enclosed cavity to receive VOCs released from the plant sample and where the VOCs can contact the colorimetric sensing elements of the sensor substrate.

For instance, Figure 5A shows exemplary sensor system **100** that comprises sensor body **110**. Sensor body **110** includes surface **119** on which is affixed array **120**. Array **120** includes colorimetric sensing components. Sensor body **110** also includes side **117** that extends perpendicularly from surface **119**. Sensor body **110** further includes a plurality of needles as exemplary cell-damaging components. Needle tip **115** extends from sensor body **110** on the same side of sensor body **110** as surface **119** and array **120** and extends beyond the end of side **117**. The needles extend through sensor body **110** such that needle base **112** is on the opposite side of sensor body **110** than needle tip **115**. When sensor system **100** is contacted with the surface of a fruit or vegetable, such as onion **130** as shown in Figure 5B, sensor body **110** forms an enclosed space to receive VOCs emitted from onion **130** after cell damage with the needles. Needle base **120** is still visible, while needle tip **115** and array **120** are hidden from view inside onion **130** or sensor body **110**, respectively.

In some embodiments, the system further comprises a detector for detecting or recording a color change and/or color change pattern of the one or more colorimetric sensing elements or the array thereof. In some embodiments, the detector comprises a camera, an optical microscope, or a smartphone reader. In some embodiments, the sensor body and/or system is provided with a handle. In some embodiments, the system is provided as a handheld or other portable device. An example detector and related configuration is disclosed in Published U.S. Patent Application No. US-2022-0236242-A1, published July 28, 2022, incorporated herein by reference in its

entirety. Thus, the system can be used *in-situ*, i.e., at a location where a fruit or vegetable is grown, stored, or sold.

5 In some embodiments, the presently disclosed subject matter provides a method of detecting one or more VOCs of interest in a plant. In some
embodiments, the method comprises: (i) contacting a plant sample with a cell-
damaging component configured to damage at least one plant cell in the plant
sample and induce release (initiate release or increase the rate of release) of
one or more VOCs of interest from the plant sample; (ii) providing one or more
10 colorimetric sensing elements, wherein one or more of the one or more
colorimetric sensing agents undergo a color change when one or more VOCs
of interest are released by the contacting of (i); and (iii) detecting a color
change associated with one or more of the one or more colorimetric sensing
elements, thereby detecting one or more VOCs of interest. For instance, a
change in color of one or more of the colorimetric sensing elements or a
15 pattern of color change in a plurality of colorimetric sensing elements (e.g., an
order of color change in a plurality of colorimetric sensing elements) can
provide information regarding the presence or absence of one or more VOCs
of interest or semi-quantitative information regarding the relative amount of
one or more VOCs of interest.

20 In some embodiments, the plant sample is from a fruit or a vegetable
(e.g., an edible part of a fruit or vegetable). In some embodiments, the one or
more VOCs of interest comprise one or more aldehyde, ketone, or sulfur-
containing VOCs. In some embodiments, the one or more VOCs of interest
are associated with a species of fruit or vegetable. Additionally or
25 alternatively, in some embodiments, the one or more VOCs of interest are
associated with a sensory property of the fruit or vegetable (e.g., a flavor
profile and/or ripeness).

In some embodiments, the fruit or vegetable is an *Allium* plant or a part
thereof (e.g., a leaf or a bulb). In some embodiments, the *Allium* plant is an
30 onion (*Allium cepa*) plant or a part thereof, e.g., an onion bulb or leaf. In some
embodiments, the one or more VOCs of interest comprise one or more
aldehyde, ketone, or sulfur-containing VOCs associated with a sensory
property of an *Allium* plant, such as, but not limited to sweetness/pungency or
tearing intensity.

In some embodiments, the fruit or vegetable is selected from melon, carrot, watermelon, and tomato or a part thereof, such as a leaf or flesh. In some embodiments, the one or more VOCs of interest comprise one or more aldehyde, ketone, or sulfur-containing VOCs associated with a species of a fruit or vegetable plant or with a sensory property of such plant, such as, but not limited to, sweetness and/or flavor. In some embodiments, detecting the one or more VOCs associated with sweetness and/or flavor can be used to predict flavor and/or eating quality. In some embodiments, the method can further comprise detecting the sugar content of the plant sample (e.g., via refractometer).

In some embodiments, the contacting can comprise puncturing the surface of the plant sample, e.g., to damage one or more cells in the sample. In some embodiments, the puncturing is performed using one or more needles or one or more microneedles. The length of the needles or the tip end of the needles can vary depending upon the type of plant sample and/or plant sample penetration depth desired. In some embodiments, the puncturing is performed to penetrate the plant sample to a depth of at least about 5 mm or at least about 10 mm. In some embodiments, the puncturing is performed to penetrate the plant sample to a depth of at least about 15 mm, at least about 20 mm, at least about 25 mm, at least about 30 mm, at least about 35 mm, at least about 40 mm, at least about 45 mm, or at least about 50 mm. In some embodiments, e.g., when the plant sample is an onion bulb, the puncturing is performed to penetrate to the second, third, fourth, or fifth scale of the bulb. In further embodiments (e.g., for plant samples with thicker skin and/or rinds), the puncturing can be performed at a higher depth (e.g., depending on skin thickness) to a depth of up to or at least about 2.5 cm.

In some embodiments, the one or more colorimetric sensing elements comprises a plurality of colorimetric sensing elements (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or more colorimetric sensing elements). In some embodiments, the colorimetric sensing elements are provided in an array (e.g., printed in individual spots on a substrate, such as paper or nitrocellulose, in a pattern).

In some embodiments, the detecting comprises analyzing a color change profile (or pattern) in the array. The color change profile or pattern

can be associated with which element or elements of the plurality of colorimetric sensing elements in the array change color over a particular VOC exposure time period (e.g., 2 minutes, 5 minutes, 10 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes, etc.) and/or with a temporal order of color change of at least two or more colorimetric sensing elements in the array. The VOC exposure time period associated with a particular profile can, in some embodiments, be associated with a particular plant sample type and/or with a particular device design (e.g., based on the distance between the sensor array and the plant sample and/or the containment level of the VOCs). In further embodiments, the color change profile or pattern can also be associated with VOC composition, that is, which VOC is more abundant at a given exposure time. In some embodiments, the analyzing further comprises comparing the color change profile in the array to a color change profile provided by performing the method on a control plant sample having one or more known properties. For example, the color change profile of an onion bulb sample can be compared to the color change profiles of onion bulbs of known tearing intensity (e.g., analyzed by one or more additional methods, such as GC-MS).

In some embodiments, the detecting is performed visually. Thus, in some embodiments, color change of the one or more colorimetric sensing elements can be assessed by the human eye and/or compared visually to color change associated with a known sample. Alternatively or additionally, the detecting can be performed using a camera, an optical microscope, or a smartphone reader. In some embodiments, the color change can be quantified. For example, RGB values can be measured. In some embodiments, the color change is a change in RGB value (ΔRGB). ΔRGB can be determined based comparing RGB values prior to VOC exposure and after VOC exposure. Thus, in some embodiments, detecting comprises creating a difference map that illustrates the color differences between a colorimetric sensing element array prior to exposure to VOCs (i.e., a reference array) and after exposure to the VOCs. From the one or more images of the array, a matrix of RGB values can be calculated (e.g., by a computer processor) for each array element and the differences (ΔR , ΔG , ΔB) between the values for each array element can be calculated to arrive at the difference

map. In addition, the Euclidean Distance, defined as $ED = \sqrt{\Delta R^2 + \Delta G^2 + \Delta B^2}$, can be calculated for each array element for quantitative analysis. The ED values of each element can form a unique sensor response pattern for each VOC or VOC combination. For classification, the ED response pattern can be recognized and differentiated by principal component analysis (PCA). Although this is one possible method for determining color change, those having ordinary skill in the art will appreciate that any possible color comparison algorithm or other method such as machine learning is capable of being utilized as well.

In some embodiments, the plant sample is a sample present at a site where the plant is grown (e.g., on a farm, in an orchard or vineyard), stored (e.g., in a warehouse) or sold (e.g., in a supermarket, restaurant, or other site where the plant or a product prepared therefrom is sold). In some embodiments, the plant sample can be a sample being transported from a growing site to a consumption or distribution site. In some embodiments, the plant sample can be present at a site related to the end use of the plant sample (e.g., consumption), such as in a restaurant or residence. Thus, in some embodiments, the method is performed at a site where the plant is grown, stored, or sold, rather than in a laboratory.

In some embodiments, the presently disclosed subject matter provides a method of selecting an *Allium* plant having a desired tearing intensity and/or pungency or based on tearing intensity and/or pungency. In some embodiments, the method comprising: (i) damaging at least one cell of an *Allium* plant, wherein the damaging induces release of a plurality of volatile organic compounds (VOCs) from the damaged cell of the *Allium* plant but does not impact the viability of the *Allium* plant; (ii) contacting the plurality of VOCs with an array comprising a plurality of colorimetric sensing elements; (iii) detecting a color change profile in the array, wherein detecting the color change profile comprises determining which of the plurality of colorimetric sensing elements in the array change color over a pre-determined time period and/or determining a temporal order of color change of at least two or more colorimetric sensing elements in the array; and (iv) determining the tearing intensity and/or pungency of the *Allium* plant based on the color change profile

of step (iii). In some embodiments, the *Allium* plant is selected from the group including, but not limited to, an onion plant, a shallot plant, a leek plant, and a garlic plant. In some embodiments, the *Allium* plant is an onion plant. In some embodiments, the at least one cell of the *Allium* plant is at least one cell in an onion bulb.

The damaging can be performed by any suitable method. In some embodiments, the damaging comprises puncturing the *Allium* plant (e.g. with one or more needles, tacks or hole punches); penetrating or cutting the *Allium* plant (e.g., with a knife or bladed instrument); by grinding a portion of the *Allium* plant (e.g. with a blender or similar instrument); or by crushing a portion of the *Allium* plant (e.g., with a mortar and pestle).

The pre-determined time period can depend upon a number of factors, including, but not limited to, the colorimetric sensing elements used in the array, the distance between the array and the damaged cell or cells, if the array and/or damaged plant cells are in an open or an enclosed space, the number of damaged cells. In some embodiments, the pre-determined time period is less than about 60 minutes, less than about 45 minutes, less than about 30 minutes, less than about 20 minutes, less than about 15 minutes, less than about 10 minutes, or less than about 5 minutes. In some embodiments, the pre-determined time period is about 2 minutes.

In some embodiments, step (iv) comprises comparing the color change profile from (iii) with a color change profile of one or more control *Allium* plants of known tearing intensity (e.g., where the control *Allium* plants have an assigned tearing intensity in the 1-7 tearing scale of sensory tearing intensity assessment methods). In some embodiments, the method comprises selecting a tearless *Allium* plant (e.g., an *Allium* plant with a tearing intensity of 3 or less or 2 or less).

In some embodiments, the presently disclosed subject matter provides a method of developing an *Allium* plant in an *Allium* plant breeding program using a colorimetric sensing element-based method of detecting desirable traits. For example, in some embodiments, the method comprising: (i) providing a plurality of *Allium* plants; (ii) determining one or more sensory properties of each of the plurality of *Allium* plants based on color change data from of one or more colorimetric sensing elements contacted with volatile

organic compounds (VOCs) from the *Allium* plant (e.g., a color change profile from an array comprising a plurality of colorimetric sensing elements); (iii) using the one or more sensory properties determined in (ii) for crossing, selection, or advancement decisions in an *Allium* plant breeding program. In some embodiments, the color change data in step (ii) is provided using a system or method of detecting a VOC as described hereinabove. In some embodiments, step (iii) comprises: (iii-a) selecting a first *Allium* plant having a first property of interest (e.g., a low tearing intensity); (iii-b) selecting a second *Allium* plant having a second property of interest (e.g., a low pungency); (iii-c) crossing said first *Allium* plant and said second *Allium* plant to provide a hybrid *Allium* plant; optionally wherein said method further comprises (iii-d) selecting progeny of said hybrid *Allium* plant having one or more properties of interest for further breeding. In some embodiments, the hybrid *Allium* plant is a tearless *Allium* plant.

Breeding methods can include crossing, self-pollination, backcrossing, and the like, which are well-known in the art (See U.S. Patent 10,285,361, which is herein incorporated by reference in its entirety).

In some embodiments, the presently disclosed subject matter provides a computer-implemented method of determining a tearing intensity and/or pungency of an *Allium* plant, the method comprising: (i) receiving data representing a tearing intensity and/or pungency of an *Allium* plant using one or more colorimetric sensing elements (e.g., a color change profile of an array of colorimetric sensing elements contacted with volatile organic compounds (VOCs) from the *Allium* plant and/or data provided by using a system or method described herein); (ii) comparing the data from (i) to a database of data collected from colorimetric sensing elements contacted with VOCs from reference *Allium* plants, wherein the comparing is performed using a computer processor; and (iii) generating an output indicating the tearing intensity and/or pungency of the *Allium* plant based on (ii). In some embodiments, the *Allium* plant is selected from the group including, but not limited to, an onion plant, a shallot plant, a leek plant, and a garlic plant. In some embodiments, the *Allium* plant is an onion plant.

In some embodiments, the presently disclosed subject matter provides a method of selecting a melon, watermelon, carrot or tomato plant having a

desired characteristic such as sweetness or flavor. In some embodiments, the method comprising: (i) damaging at least one cell of a melon, watermelon, carrot, or tomato plant, wherein the damaging induces release of a plurality of volatile organic compounds (VOCs) from the damaged cell of the melon, watermelon, carrot, or tomato plant; (ii) contacting the plurality of VOCs with an array comprising a plurality of colorimetric sensing elements; (iii) detecting a color change profile in the array, wherein detecting the color change profile comprises determining which of the plurality of colorimetric sensing elements in the array change color over a pre-determined time period and/or determining a temporal order of color change of at least two or more colorimetric sensing elements in the array; and (iv) determining the desired characteristic of the melon, watermelon, carrot, or tomato plant based on the color change profile of step (iii). In some embodiments, the melon, watermelon, carrot, or tomato plant is selected from the group including, but not limited to, a honeydew plant, a watermelon plant, a cantaloupe plant, a carrot plant, and a tomato plant.

The damaging can be performed by any suitable method. In some embodiments, the damaging comprises puncturing the melon, watermelon, carrot, or tomato plant (e.g. with one or more needles, tacks or hole punches), by cutting the melon, watermelon, carrot, or tomato plant (e.g., with a knife or bladed instrument); by grinding a portion of the melon or plant (e.g. with a blender or similar instrument); and/or by crushing a portion of the melon, watermelon, carrot, or tomato plant (e.g., with a mortar and pestle). In some embodiments, e.g., for fruits with relatively thicker rinds, such as melon, the contacting can comprise cutting the plant sample and detecting VOCs emitted from the cut flesh. In some embodiments, the cut flesh is placed in a closed container with a colorimetric dye array (e.g., within minutes or seconds of the cutting). In some embodiments, the damaging does not impact the viability of the melon, watermelon, carrot, or tomato plant. In some embodiments, the damaging impacts the viability of the melon, watermelon, carrot, or tomato plant.

In some embodiments, the presently disclosed subject matter provides a method of developing a melon, watermelon, carrot, or tomato plant in a melon, watermelon, carrot or tomato plant breeding program using a

colorimetric sensing element-based method of detecting desirable traits. For example, in some embodiments, the method comprising: (i) providing a plurality of melon, watermelon, carrot, or tomato plants; (ii) determining one or more sensory properties of each of the plurality of melon, watermelon, carrot, or tomato plants based on color change data from one or more colorimetric sensing elements contacted with volatile organic compounds (VOCs) from the melon, watermelon, carrot, or tomato plant (e.g., a color change profile from an array comprising a plurality of colorimetric sensing elements); (iii) using the one or more sensory properties determined in (ii) for crossing, selection, or advancement decisions in a melon, watermelon, carrot, or tomato plant breeding program. In some embodiments, the color change data in step (ii) is provided using a system or method of detecting a VOC as described hereinabove. In some embodiments, step (iii) comprises: (iii-a) selecting a first melon, watermelon, carrot, or tomato plant having a first property of interest (e.g., a sweetness and/or flavor); (iii-b) selecting a second melon, watermelon, carrot, or tomato plant having a second different property of interest (e.g., sweetness and/or flavor); (iii-c) crossing said first melon, watermelon, carrot, or tomato plant and said second melon, watermelon, carrot, or tomato plant to provide a hybrid melon, watermelon, carrot, or tomato plant; optionally wherein said method further comprises (iii-d) selecting progeny of said hybrid melon, watermelon, carrot, or tomato plant having one or more properties of interest for further breeding.

In some embodiments, the presently disclosed subject matter provides a computer-implemented method of determining sweetness and/or flavor of a melon, watermelon, carrot, or tomato plant, the method comprising: (i) receiving data representing a determined level of sweetness and/or flavor of a melon, watermelon, carrot, or tomato plant using one or more colorimetric sensing elements (e.g., a color change profile of an array of colorimetric sensing elements contacted with volatile organic compounds (VOCs) from the melon, watermelon, carrot, or tomato plant and/or data provided by using a system or method described herein); (ii) comparing the data from (i) to a database of data collected from colorimetric sensing elements contacted with VOCs from reference melon, watermelon, carrot, or tomato plants, wherein the comparing is performed using a computer processor; and (iii) generating

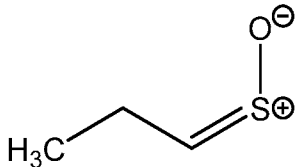
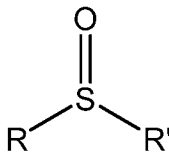
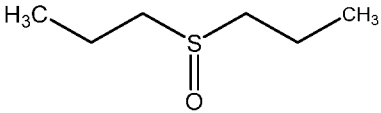
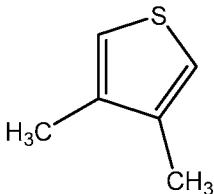
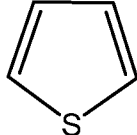
an output indicating the sweetness and/or flavor of the melon, watermelon, carrot, or tomato plant based on (ii). In some embodiments, the melon or tomato plant is selected from the group including, but not limited to, a honeydew plant, a watermelon plant, a cantaloupe plant, a carrot plant, or a tomato plant.

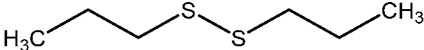
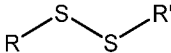
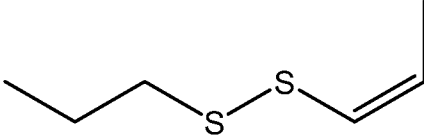
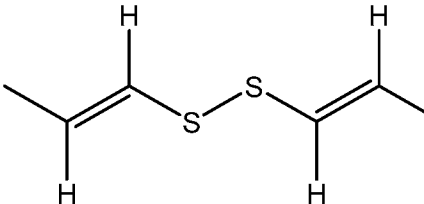
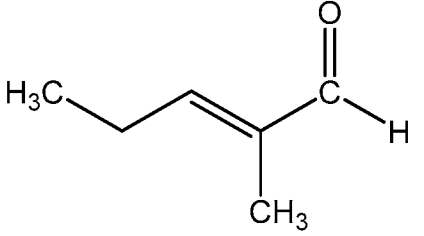
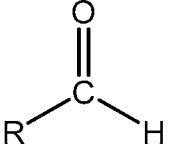
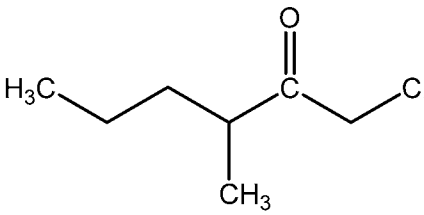
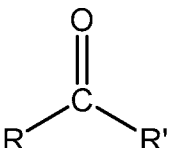
EXAMPLE 1

Screening of Colorimetric Dyes using Pure VOCs

Colorimetric dyes were screened against available onion VOCs including non-sulfur VOCs (e.g., aldehydes and ketones) and sulfur-containing VOCs (e.g., sulfoxide, thiophenes, and disulfides). VOCs tested include 2-methyl 2-pentenal (an exemplary aldehyde), 2-methyl-3-heptanone (an exemplary ketone), thiopropanal sulfoxide and dipropyl sulfoxide (exemplary sulfoxides), 3,4-dimethylthiophene (an exemplary thiophene), and dipropyl disulfide (an exemplary disulfide. See Table 1, below.

Table 1. Exemplary Onion VOCs.

Sulfur/ Non- Sulfur Type	Volatile	Chemical Structure	Functional Group
Sulfur- contain- ing volatiles	Thiopro- panal sulfoxide (lachrymator y factor or LF)		Sulfoxide 
	Dipropyl sulfoxide		
	3,4- Dimethyl- thiophene		Thiophene 

	Dipropyl disulfide		Disulfide 
	Propenyl propyl disulfide		
	Di-1-propenyl disulfide		
Non-sulfur containing volatiles	2-Methyl-2-pentenal		Aldehyde/ 
	4-Methyl-3-heptanone		Ketone 

Colorimetric dyes were printed on a nitrocellulose acetate membrane (as an exemplary substrate material) to provide a “colorimetric strip”. Briefly, 500 µl of a sol-gel solution (optionally comprising an acid (e.g., hydrochloric acid (HCl), base (e.g., sodium hydroxide (NaOH) or tetrabutylammonium hydroxide (TBAH)), amine (e.g., pyrrolidone), or zinc salt) was added to each dye. Using pins (VNP Scientific) capable of delivering about 100 nL of dye-containing solution, the array was printed on a nitrocellulose acetate membrane. Printed membranes were placed in a 60°C oven for 24 hours, after which the oven temperature was reduced to 35°C and the arrays left for an additional 24 hours. Arrays were stored under nitrogen until use.

Pure VOCs and a colorimetric strip were placed inside a closed vial to expose the colorimetric dyes to the VOC. See Figure 1. Assessment of color change was performed after 60 minutes. Dyes were selected based on the

following criteria: a positive response (i.e., color change with R-G-B- values > 3 (i.e., greater than background threshold change generated by exposure of the dye to air); lack of reaction between the dye and the colorimetric strip substrate material (i.e., the nitrocellulose acetate membrane); and lack of precipitate or particulate formation when the dye is printed on the colorimetric strip substrate material. Initial studies with 1-pentanethiol indicated that the limit of detection (LOD) of colorimetric dyes printed on a colorimetric strip was about 25 parts-per-million (ppm). Studies with 2-methyl-2-pentenal indicated a LOD of about 10 ppm.

The color change response of exemplary colorimetric dyes to different sulfur-containing or non-sulfur containing commercially available pure VOCs after 60 minutes of exposure are shown in Tables 2A-2E, below. 2-Methyl-2-pentenal, 4-methyl-3-heptanone, dipropyl disulfide, and 3,4-dimethylthiophene have been found in onions via VOC assessment based on SPME-GC-MS and GC-MS liquid injections. The commercially available 2-methyl-3-heptanone is used as a proxy for the 4-methyl-3-heptanone found in onions.

Table 2A. Responsiveness of Colorimetric Dyes to Pure VOCs (part 1).

Volatile Name	Target Functional Group	Reichardt's dye + sol gel	Mero-cyanine 540 + sol gel	1-Ethyl-4-(2-hydroxystyryl)-pyridinium iodide + sol gel	Bromo-pyrogallol red + sol gel	Methyl red + sol gel + NaOH
2-Methyl 2-pentenal**	Aldehyde	X	X	X		X
E-(2)-hexenal	Aldehyde	X	X	X		
2-Methyl-3-heptanone ^a	Ketone	X	X	X		
Acetone	Ketone	X	X	X		X
1-Octen-3-ol	Alcohol	X	X	X		
Amyl mercaptan	Thiol	X				

(1-Pentane-thiol)						
Propyl sulfoxide	Sulfoxide		X			
Diethyl sulfide	Sulfide				X	
Dipropyl disulfide**	Disulfide		X			
3,4-Dimethyl-thiophene**	Thiophene	X	X			

** found in onion based on SPME-GC-MS and GC-MS liquid injection; ^a proxy for 4-methyl-3-heptanone identified in onion via SPME-GC-MS.

Table 2B. Responsive of Colorimetric Dyes to Pure VOCs (part 2).

Volatile Name	Target Functional Group	Thymol blue + sol gel	Bromo-phenol red + sol gel	Nitrazine Yellow + sol gel + NaOH	Pyro-cathecol violet + sol gel	Cresol Red + sol gel
2-Methyl 2-pentenal**	Aldehyde			X	X	
E-(2)-hexenal	Aldehyde			X	X	
2-Methyl-3-heptanone ^a	Ketone					
Acetone	Ketone			X	X	X
1-Octen-3-ol	Alcohol					
Amyl mercaptan (1-Pentanethiol)	Thiol					
Propyl sulfoxide	Sulfoxide					
Diethyl sulfide	Sulfide	X	X			
Dipropyl disulfide**	Disulfide					
3,4-Dimethyl-thiophene**	Thiophene					

5 ** found in onion based on SPME-GC-MS and GC-MS liquid injection; ^aproxy for 4-methyl-3-heptanone found in onions via SPME-GC-MS.

Table 2C. Responsiveness of Colorimetric Dyes to Pure VOCs (part 3).

Volatile Name	Target Functional Group	Zinc-tetraphenyl-porphyrin-amine + pyrrolidone (amine) + chloroform	Tetrakis-(2,4,6-trimethyl-phenyl)porphyrinato-cobalt(II) amine + pyrrolidone (amine) + chloroform	Tetrakis-(pentafluorophenyl)-porphyrine Iron(III) chloride amine + pyrrolidone (amine) + chloroform	Cresol red + pyrrolidone (Amine)	Methyl Red + sol gel + NaOH
2-Methyl-2-pentenal**	Aldehyde	X	X		X	X
E-(2)-hexenal	Aldehyde					
2-Methyl-3-heptanone ^a	Ketone	X			X	
Acetone	Ketone				X	X
1-Octen-3-ol	Alcohol					
Amyl mercaptan (1-Pentanethiol)	Thiol	X	X	X		
Propyl sulfoxide	Sulfoxide					
Diethyl sulfide	Sulfide	X	X	X	X	
Dipropyl disulfide**	Disulfide					
3,4-Dimethyl-thiophene**	Thiophene	X			X	

** found in onion based on SPME-GC-MS and GC-MS liquid injection; ^aproxy for 4-methyl-3-heptanone found in onions via SPME-GC-MS.

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Table 2D. Responsiveness of Colorimetric Dyes to Pure VOCs (part 4).

Volatile Name	Target Functional Group	Nitrazine	Cresol red + sol gel	Parasol-aniline	Zn(OAc) ₂ + m-cresol	Zn(OAc) ₂ + bromocresol
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		Yellow + sol gel + NaOHH	+ NaOH	hydro- chloride + HCl + form- aldehyde + H ₂ O	purple + sol gel + TBAH	green + sol gel + TBAH
2-Methyl 2-pentenal**	Aldehyde	X		X		X
E-(2)-hexenal	Aldehyde	X		X	X	X
2-Methyl-3- heptanone ^a	Ketone					X
Acetone	Ketone	X	X		X	
1-Octen-3-ol	Alcohol					X
Amyl mercaptan (1- Pentanethiol)	Thiol					
Propyl sulfoxide	Sulfoxide					
Diethyl sulfide	Sulfide		X		X	
Dipropyl disulfide**	Disulfide					
3,4-Dimethyl- thiophene**	Thiophene					

** found in onion based on SPME-GC-MS and GC-MS liquid injection; ^aproxy for 4-methyl-3-heptanone found in onions via SPME-GC-MS.

Table 2E. Responsiveness of Colorimetric Dyes to Pure VOCs (part 5).

Volatile Name	Target Functional Group	Zn(OAc) ₂ + bromo- phenol blue + sol gel + TBAH	Reichardt's Dye + sol gel	Mero- cyanin 540 + sol gel	1-ethyl- 4(2- hydroxy- styryl)- pyridinium iodide + sol gel	Bromo- pyrogallol red + sol gel
2-Methyl 2-pentenal**	Aldehyde	X	X	X	X	
E-(2)-hexenal	Aldehyde	X	X	X	X	

2-Methyl-3-heptanone ^a	Ketone	X	X	X	X	
Acetone	Ketone	X	X	X	X	
1-Octen-3-ol	Alcohol	X	X	X	X	
Amyl mercaptan (1-Pentanethiol)	Thiol	X	X	X	X	
Propyl sulfoxide	Sulfoxide			X		
Diethyl sulfide	Sulfide	X	X	X		X
Dipropyl disulfide**	Disulfide			X		
3,4-Dimethyl-thiophene**	Thiophene		X	X		

** found in onion based on SPME-GC-MS and GC-MS liquid injection; ^aproxy for 4-methyl-3-heptanone found in onion via SPME-GC-MS.

5 Colorimetric dye responsiveness to pure onion-related VOCs is summarized in Table 3, below. As the pure onion VOC testing was performed at saturated concentrations, the VOC concentrations are potentially higher than the actual concentrations in onions. Nonetheless, based on these results, 16 colorimetric dyes were selected for an exemplary colorimetric dye array for use in VOC testing in real onion samples. See Table 4, below, which lists the dye solutions used to prepare the array. These 16 dyes were also assigned a dye identification number from 1 to 16 for future reference in onion sampling.

15 Table 3. Summary of Exemplary Colorimetric Dye Responsiveness to Pure Onion-Related VOCs.

ID	Colormetric Dye	2-methyl-2-penten-al	4-methyl-3-heptanone**	Dipropyl Sulfoxide	Di-propyl disulfide	3,4-dimethyl thiophene
1	Zinc-tetraphenylporphyrin	X	X			X

	amine + pyrrolidone (amine) + chloroform					
2	Tetrakis(2,4,6-trimethylphenyl)- porphyrinatocobalt(II) amine + pyrrolidone (amine) + chloroform	X				
4	Cresol red + pyrrolidone (amine)	X	X			X
5	Methyl red + sol gel + NaOH	X				
6	Nitrazine yellow + sol gel + NaOH	X				
8	Parasoaniline hydrochloride + HCl + Formaldehyde + H ₂ O	X				
10	Zn(OAc) ₂ + Bromophenol green + sol gel + TBAH	X	X			
11	Zn(OAc) ₂ + Bromophenol blue + sol gel + TBAH	X	X			
12	Reichardt's dye + sol gel	X	X			X
13	Merocyanin 540 + sol gel	X	X	X	X	X
14	1-Ethyl-4-(2-hydroxystyryl)pyridinium iodide + sol gel	X	X			
15	Pyrocathocol violet + sol gel	X				

** 4-Methyl-3-heptanone responsiveness was tested for using a similar commercially available ketone, i.e., 2-Methyl-3-heptanone.

5 Table 4. Colorimetric Dyes Selected for Exemplary Onion Sensor Dye Strip and Summary of Exemplary VOC Responsiveness.

ID	Colorimetric Dye	Dye Type	Target Func-	2-meth-yl-2-	4-methyl-3-	Di-Prop-yl	Di-pro- pyl	3,4-di-methyl
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			tional Group	pent- enal	hepta- none	Sulf- oxide	di- sul- fide	thio- phene
1	Zinc-tetraphenyl- porphyrin amine + pyrrolidone (amine) + chloroform	Por- phyrin Amine	Sulf- oxide, Alde- hyde, Ketone	X	X			X
2	Tetrakis(2,4,6- trimethylphenyl)por- phyrinatocobalt(II) amine + pyrrolidone (amine) + chloroform			X				
3	Tetrakis(pentafluoro- phenyl)porphine iron (III)-chloride amine + pyrrolidone (amine) + chloroform							
4	Cresol red + pyrrolidone (amine)	Amine- based		X	X			X
5	Methyl red + sol gel + NaOH	Acid Indi- cators		X				
6	Nitrazine yellow + sol gel + NaOH			X				
7	Cresol red + sol gel + NaOH							
8	Parasoaniline hydrochloride + HCl + formaldehyde + H ₂ O	Amine- based		X				
9	Zn(OAc) ₂ + m-Cresol purple + sol gel + TBAH	Metal salt	Thiol					
10	Zn(OAc) ₂ + Bromophenol green + sol gel + TBAH			X	X			
11	Zn(OAc) ₂ + Bromophenol blue + sol gel + TBAH			X	X			
12	Reichardt's dye + sol gel	Solvato- chromic	Sulfide,	X	X			X

13	Merocyanin 540 + sol gel		Alde- hyde, Ketone, Alcohol	X	X	X	X	X
14	1-Ethyl-4-(2-hydroxystyryl)pyridinium iodide + sol gel			X	X			
15	Pyrocathocol violet + sol gel	Base Indi- cators	Alde- hyde	X				
16	Cresol red + sol gel							

EXAMPLE 2

Open-Air Sampling of Onions with Colorimetric Dyes

Four onion genotypes were selected for assessment of tearing intensity via open-air sampling and using colorimetric dyes. Two of the selected genotypes (A and B) are considered as tearing, while the other two (C and D) are considered tearless.

The open-air sampling method is illustrated in Figure 2. Briefly, the 16 colorimetric dyes selected as described in Example 1 were printed in a 4x4 array on a nitrocellulose acetate membrane. Onions of each of the four genotypes were peeled, chopped and placed in a small, uncovered petri dish. The colorimetric dye arrays were placed together with an onion-containing petri dish in a larger, covered petri dish to expose the dyes to VOCs from the cut onion. The pattern of dye color change in the array was determined at four different exposure times: 15 minutes, 30 minutes 1 hour, and 16 hours. Differentiation of colorimetric dye response to tearing versus tearless onions can potentially include: different dyes responding to tearing versus tearless onions; the same colorimetric dye responding to both tearing and tearless onions, but at different response times; or the same colorimetric dye responding to tearing and tearless onions at the same exposure time, but showing different R-G-B values.

Results from the open-air sampling studies are shown visually in Figures 3A-3D and summarized in Tables 5-7, below. There was a lack of differentiation in colorimetric dye response patterns (dyes 4 and 7-16) between tearing and tearless onions after extended exposure times (16 hours).

Table 5. Open Air Colorimetric Dye Responsiveness after 15-Minute Exposure.

Type	Onion Sample ID	Bulb #	Organic Dyes											
			4	5	6	7	8	9	10	11	12	13	15	
Tearless	D	1												
		2												
	C	1												
		2												
		3												
		4			X									
		5												
		6												
Tearing	B	1						X	X					
		2						X	X					
	A	1												
		2												
		3												
		4			X									
		5												
		6												
		7												

5

Table 6. Open Air Colorimetric Dye Responsiveness after 30 Minute Exposure

Type	Onion Sample ID	Bulb #	Organic Dyes											
			4	5	6	7	8	9	10	11	12	13	15	
Tearless	D	1												
		2												
	C	1	X							X				

Tearing		2	X		X								
		3	X										
		4	X		X								
		5											
		6										X	
	B	1						X	X				
		2						X	X				X
	A	1						X		X			
		2						X		X			
		3	X		X								
		4			X								
		5											
		6			X								
		7											

Table 7. Open Air Colorimetric Dye Responsiveness after 60 Minute Exposure.

Type	Onion Sample ID	Bulb #	Organic Dyes										
			4	5	6	7	8	9	10	11	12	13	15
Tearless	D	1	X		X	X			X	X			
		2	X		X	X			X	X			
	C	1	X					X	X	X	X	X	
		2	X		X			X	X	X		X	
		3	X		X			X	X	X		X	
		4	X		X			X	X	X		X	
		5	X						X	X			
		6	X						X	X		X	
Tearing	B	1	X	X				X	X			X	X
		2	X	X				X	X			X	X
	A	1	X					X	X	X		X	X
		2	X					X	X	X		X	

		3	X		X	X			X	X		X	
		4	X		X	X		X	X	X		X	
		5											
		6	X		X	X							
		7											

Overall, the open-air suggested a temporal pattern of colorimetric dye color change to distinguish the four onion genotypes. See Figure 4. The pattern of colorimetric dye response for tearing onions A and B started with dyes 9-11, followed by dyes 13 and 15, then dyes 4 and 5 or 4 and 6. Tearless onion D showed color change starting with dyes 4, 6, and 7 followed by dyes 10 and 11. The pattern for tearless onion C was in-between tearing onions A and B and tearless onion D. Response for onion C started with dyes 6 and 4 followed by dyes 9-11. As with onions A and B, onion C also showed response from dyes 12 and 13. The results further suggested that the aldehyde functional group was the most reactive group for colorimetric dyes response to onion VOCs.

EXAMPLE 3

Onion Genotype Irritation Scoring and GC-MS Assessment

To further assess differences between the onion genotypes A-D from Example 2, above, more traditional testing methods were applied. For example, the genotypes were assessed via human sensory scoring. Onions were assessed for irritation on a scale of 1-7, where a score of 1 corresponds to non-noticeable irritation and 7 corresponds to strong burning or tears in the eyes. See Table 8, below. Additionally, for Scores 1-2, no sharp odor was detected, while for scores 3-4, a sharp (wasabi-like) odor note was present. Onions were assessed in two phases, the first after cutting the top of the onion bulb and the second after grinding onion tissue to a pulp (which typically intensifies irritation intensity).

Table 8. Human Sensory Irritation Scoring Scale for Onions.

Sensory Effect on Eye	Score
Not Noticeable -	1
Faint Burn	2
Slight Burn	3
Mild Burn	4
Moderate Burn	5
Strong Burn	6
Very Strong Burn, Tears in Eyes	7

Results are shown in Table 9, below. These results indicated that the four onion genotypes, while generally categorized as tearing or tearless could be further ranked in four different irritation intensity levels in order of irritation A>B>C>D where genotype A is the most irritating genotype and genotype D is the least irritating.

Table 9. Summary of Human Sensory Irritation Scoring of Onion Genotypes A-D.

Sample ID	Bulb Rep	Type	Cut Irritation	Blend Irritation	Avg irritation per bulb	Avg. irritation per hybrid	Irritation Scale
D-1	1	Tearless	2	2.5	2.3	1.9	2.0
D-2	2	Tearless	2	2	2.0		
D-3	3	Tearless	1	2	1.5		
C-1	1	Tearless	2.5	3.5	3.0	2.7	3.0
C-2	2	Tearless	2	3	2.5		
C-3	3	Tearless	2	3	2.5		
B-1	1	Tearing	2.5	5	3.8	3.8	4.0
B-2	2	Tearing	2.5	4.5	3.5		
B-3	3	Tearing	3	5.5	4.3		
A-1	1	Tearing	4.5	7	5.8		

A-2	2	Tearing	4	6	5.0	5.2	5.0
A-3	3	Tearing	3.5	6	4.8		

VOCs from the four onion genotypes were further analyzed semi-quantitatively via SPME-GC-MS. See Tables 10A-10C, below.

5 Table 10A. VOC Profiles for Tearing versus Non-Tearing Onions.

VOC	VOC Functional Group	Ratio of Peak Area Tearing/Non-Tearing
Decanal	Aldehyde	<1
2-Methyl-2-Pentenal		≥ 5x (up to 20x)
4-Methyl-2-pentenal		≥ 5x (up to 20x)
3,4-Dimethyl-2,5-dihydrothiophene-2-one	Ketone	≥ 1.5-2x
4-Methyl-3-heptanone		≥ 3-4x
2,4-Dimethyl-3-hexanol	Alcohol	<1
3-Mercapto-2-methylpropanol		≥ 1.5-2x
1-[2-(Isobutyroxy)-1-methylethyl]-2,2-dimethylpropyl 2-methylpropanoate	Ester	**
2-Hydroxymethylcyclopropanecarboxylic acid methyl ester		**
<i>trans</i> -2-Hydroxycyclohexanyl acetate		**
1,5-Hexadien-3-ol acetate		≥ 5x (up to 20x)
Dipropyl disulfide	Disulfide	<1
<i>trans</i> -Propenyl propyl disulfide		<1
<i>cis</i> -Di-1-propenyl disulfide		<1
<i>trans</i> -Di-1-propenyl disulfide		<1
1-Propyl-2-(thiohept-2-en-5-yl) disulfide		<1

Diallyl disulfide		$\cong 1$
Methyl- <i>trans</i> -propenyl disulfide		$\geq 3-4x$
Propyl hexyl disulfide		$\geq 3-4x$
Methyl- <i>cis</i> -propenyl disulfide		$\geq 5x$ (up to 20x)
Dipropyl trisulfide	Trisulfide	<1
<i>cis</i> -Propenyl propyl trisulfide		<1
Di-1-propenyl trisulfide		<1
<i>trans</i> -Propenyl propyl trisulfide		$\cong 1$
Dimethyl trisulfide		**
Methyl propyl trisulfide		**
<i>cis</i> -Methyl propenyl trisulfide		$\geq 5x$ (up to 20x)
<i>trans</i> -Methyl propenyl trisulfide		$\geq 5x$ (up to 20x)
Di-1-propenyl tetrasulfide	Tetrasulfide	**
Dimethyl tetrasulfide		**
n-Decyl sulfide	Sulfide	**
2-(Methylthio)propane		$\geq 1.5-2x$
Dithio(1-propenyl)propionate		$\geq 1.5-2x$
Hexyl vinyl sulfide		**
<i>trans</i> -Zwiebelane		$\geq 3-4x$
<i>cis</i> -Zwiebelane		$\geq 3-4x$
Allyl thiopropionate		$\geq 5x$ (up to 20x)
<i>cis</i> -2-Ethyl-3-methylthiophane	Thiophane/ Thiophene	**
2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene		<1
<i>trans</i> -2-Ethyl-3-methylthiophane		**
2-Acetylthiophene		**
3,4-Dimethyl-2,5-dihydrothiophene-2-one		**
3,4-Dimethylthiophene		$\geq 3-4x$

2-Methyl-5-(methylsulfanyl)thiophene		**
2-Mercapto-3,4-dimethyl-2,3-dihydrothiophene	Thiol	<1
3-Mercapto-2-methylpropanol		≥ 1.5-2x
Isobutyl isothiocyanate		≥ 1.5-2x
Dipropyl sulfoxide	Sulfoxide	<1
Thiopropenal sulfoxide		≥ 1.5-2x
<i>trans</i> -Zwiebelane		≥ 3-4x
<i>cis</i> -Zwiebelane		≥ 3-4x
4,6-Diethyl-1,2,3,5-tetrathiolane	Thiolane	< 1
5,7-Diethyl-1,2,3,4,6-pentathiepane		≅ 1
1,2-Dithiolane		≥ 1.5-2x
1,2-Dithiolane-1,1-dioxide		**
4-Methyl-3H-1,2-dithiol-3-thione		**
3,5-Diethyl-1,2,4-trithiolane		≥ 3-4x
<i>cis</i> & <i>trans</i> -4,6-Diethyl-1,2,3,5-tetrathiolane		**
2-Vinyl-1,3-dithiane		≥ 5x (up to 20x)
3-Vinyl-1,2-dithiacyclohex-5-ene		≥ 5x (up to 20x)
Hexathiepane		≥ 5x (up to 20x)
4-Methyl-3H-1,2-dithiol-3-one		≥ 5x (up to 20x)

** ND based on current sample size; %CV ≥ 45% among 3 bulb reps

Table 10B. Summary of VOC Differences between Tearing and Non-Tearing Onions.

Higher Concentration in Tearing Onion	Higher Concentration in Tearless Onion
2-Methyl-2-pentenal 4-Methyl-2-pentenal	

2-Vinyl-1,3-dithiane	
3-Vinyl-1,2-dithiacyclohex-5-ene	
Hexathiepane	
Allyl thiopropionate	
<i>cis</i> -Methyl propenyl trisulfide	<i>trans</i> -propenyl propyl disulfide
<i>trans</i> -Methyl-propenyl trisulfide	<i>cis</i> -Di-1-propenyl disulfide
	<i>trans</i> -Di-1-propenyl disulfide
	Dipropyl disulfide
	Di-1-propenyl trisulfide

Table 10C. Summary of SPME-GC-MS Results for Exemplary Onion VOCs of Genotypes A-D.

Volatile Name	Average Peak Area				Peak Area vs. Irritation Intensity Order (A>B>C>D)
	D	C	B	A	
Thiopropional sulfoxide	6,989,884	16,565,127	23,311,744	24,405,808	Match
2-Methyl-2-pentenal	758,380	3,795,800	8,189,717	30,090,217	Match
<i>trans</i> -Di-1-propenyl disulfide	4,833,187	4,419,830	1,563,559	5,388,915	
<i>trans</i> -Propenyl propyl disulfide	3,607,980	3,733,536	889,295	2,816,791	
Dipropyl disulfide	826,596	1,062,552	541,594	884,638	
<i>cis</i> -Di-1-propenyl disulfide	697,757	505,712	144,928	581,433	
Dipropyl sulfoxide	136,385	466,492	194,906	285,423	
3,4-Dimethylthiophene	80,449	171,588	213,410	705,557	Match
4-Methyl-2-pentenal	9,476	36,580	75,832	295,392	Match

4-Methyl-3-heptanone	7,537	18,134	34,059	47,827	Match
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These results showed that there was alignment between the irritation intensities determined via human sensory testing and the SPME-GC-MS peak areas of the VOCs thiopropanal sulfoxide, 2-methyl-2-pentenal, 3,4-dimethylthiophene, 4-methyl-2-pentenal, and 4-methyl-3-heptanone. See Table 10C. Without being bound to any one theory, these results can explain the differing dye response patterns observed in the open-air sampling studies described in Example 2, and further support the use of colorimetric dye response as a semi-quantitative approach for determining onion irritation intensity based on the temporal response pattern of the dyes. See Table 11, below.

Table 11. Summary of Temporal Colorimetric Dye Response.

Genotype	Irritation Intensity	Temporal response pattern of colorimetric dyes (open-air sampling; est. 30-min and 45-min exposure)			
A	5	9/10/11	15	13	4
B	4	9/10	15	13	4/5
C	3	6/4 or 13	9/10/11	--	--
D	2	--	6/4/7	10/11	--

EXAMPLE 4

Minimally Invasive Onion Genotype Tearing Intensity Assessment with Colorimetric Dyes – “Evaluation One”

An exemplary sensor device combining a colorimetric dye sensing component and a cell-damaging component was designed and used to analyze onion genotype tearing intensity. See Figures 5A and 5B. Onions were punctured with a device comprising four needles and the needles were left inserted. See Figure 5B. Alternatively, onions were punctured with a single needle and the needle removed. Figure 6 shows the colorimetric dye response pattern of a tearing onion after exposure times of 2 minutes, 5

minutes, 15 minutes, or 30 minutes; as well as the different colorimetric dye response pattern between a tearing onion genotype and a tearless onion genotype after 2 minutes of exposure, indicating that within 2 minutes of exposure, the colorimetric sensor differentiated tearing onion from the tearless genotype.

Overall, there was a very fast reaction between the colorimetric dyes and the onion VOCs in the close-air sampling provided by the exemplary device, although this resulted in some loss of order in the colorimetric dye response.

Figures 7A and 7B compare colorimetric dye response patterns from a sample size of 52 onions, 26 tearing onions of the same genotype and 26 tearless onions of the same genotype. Each onion bulb was tested in duplicate, using a two-minute exposure time. Nineteen of the same onion samples were also assessed by a blind human sensory test for an exposure time of 10-15 seconds. Sensory testing indicated that bulb numbers 13, 14, 15, 17, 21, 22, 25 were tearless onions and that bulb numbers 16, 18, 19, 20, 23, 24, 26 were not tearless onions.

EXAMPLE 5

Minimally Invasive Onion Genotype Tearing Intensity Assessment with Colorimetric Dyes – “Evaluation Two”

The exemplary sensor device combining a colorimetric dye sensing component and a cell-damaging component shown in Figures 5A and 5B was used to analyze onion genotype tearing intensity in a sample size of 100 individual onions bulbs (40 tearing and 60 tearless). The tearless onions were selected from 6 different tearless onion genotypes (referred to herein as E, F, G, H, I, and J) and the tearing onions from 4 different tearing genotypes (referred to herein as K, L, M, and N). The onion bulbs were punctured with a device comprising four needles and the needles were left inserted. See Figure 5B for example. The colorimetric dyes used in the sensor are the same as those described in Table 4, above.

Figures 8A-8E show the colorimetric dye response pattern of the tearless and tearing onion genotypes after an exposure time of 2 minutes. Overall, there was a very fast reaction between the colorimetric dyes and the

onion VOCs in the close-air sampling provided by the exemplary device. Table 12, below, shows which onions in the 100-onion sample set were tearing and which were tearless and the genotype (E-N) for each onion.

5 Table 12. Onion Tearing Intensity for the Onions in Figures 8A-8E.

Sample No.	Tearing or Tearless	Genotype		Sample No.	Tearing or Tearless	Genotype
1	Tearless	J		51	Tearing	M
2	Tearing	N		52	Tearing	L
3	Tearing	K		53	Tearless	F
4	Tearless	F		54	Tearless	G
5	Tearless	H		55	Tearless	E
6	Tearless	G		56	Tearing	N
7	Tearless	E		57	Tearing	K
8	Tearless	I		58	Tearless	J
9	Tearing	M		59	Tearless	I
10	Tearing	L		60	Tearless	H
11	Tearing	K		61	Tearless	F
12	Tearless	J		62	Tearless	G
13	Tearless	H		63	Tearing	M
14	Tearless	E		64	Tearless	H
15	Tearless	G		65	Tearless	I
16	Tearing	M		66	Tearing	K
17	Tearless	I		67	Tearing	L
18	Tearing	N		68	Tearless	E
19	Tearing	L		69	Tearless	J
20	Tearless	F		70	Tearing	N
21	Tearless	H		71	Tearing	L
22	Tearless	F		72	Tearless	J
23	Tearless	G		73	Tearing	K
24	Tearless	I		74	Tearing	N
25	Tearing	L		75	Tearless	E

26	Tearless	J		76	Tearless	G
27	Tearless	E		77	Tearless	I
28	Tearing	K		78	Tearless	F
29	Tearing	N		79	Tearless	H
30	Tearing	M		80	Tearing	M
31	Tearless	I		81	Tearless	G
32	Tearless	G		82	Tearless	H
33	Tearless	H		83	Tearless	E
34	Tearing	L		84	Tearing	N
35	Tearless	J		85	Tearing	M
36	Tearing	K		86	Tearless	J
37	Tearless	F		87	Tearless	I
38	Tearless	E		88	Tearing	K
39	Tearing	M		89	Tearless	F
40	Tearing	N		90	Tearing	L
41	Tearing	N		91	Tearless	E
42	Tearing	L		92	Tearless	F
43	Tearless	H		93	Tearing	M
44	Tearing	M		94	Tearless	J
45	Tearing	K		95	Tearing	L
46	Tearless	F		96	Tearless	G
47	Tearless	J		97	Tearless	H
48	Tearless	E		98	Tearing	N
49	Tearless	I		99	Tearing	K
50	Tearless	G		100	Tearless	I

Table 13, below, summarizes the human sensory irritation scoring system using a scale of 1-7, indicating the average irritation level of each of the onion genotypes E-J (tearless) and K-N (tearing), while Table 14, below, shows the percentage of human sensory irritation of the eye, nose and throat for each onion genotype. There is a clear separation of the intensity between the tearing and tearless onions.

Table 13. Human Sensory Irritation Scoring Scale and Average for Onion Genotypes E-N.

Sensory Effect on Eye	Score	Average Score for Genotypes E-N
Not Noticeable	1	
Faint Burn	2	E
Slight Burn	3	F, G, H, I, J
Mild Burn	4	
Moderate Burn	5	K, L
Strong Burn	6	M, N
Very Strong Burn, Tears in Eyes	7	

Table 14. Percentage (%) of Bulbs with Irritation During Cutting.

	Onion Genotype	Eye Irritation	Nose Irritation	Throat Irritation
Tearless	E	0%	60%	70%
	F	10%	50%	40%
	H	0%	60%	70%
	I	0%	40%	80%
	G	0%	80%	60%
	J	10%	80%	40%
Tearing	K	30%	90%	70%
	L	50%	90%	40%
	M	40%	90%	80%
	N	60%	90%	70%

5

Table 15, below, summarizes colorimetric dye responsiveness from Evaluation Two after 2 minutes of exposure time for the tearless onion genotypes. Results indicated that tearless genotypes had negative responses for colorimetric dyes 1-3 and less response for colorimetric dyes 12-16.

10

Table 15. Dye Responsiveness for Tearless Genotypes.

	Adj. Avg. Irrita- -tion	Colorimetric Dye															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
E	1.2				X		X		X	X	X	X	X			X	
F	1.3				X	X			X	X	X	X		X	X	X	X
H	1.7				X			X	X	X	X	X			X	X	X
I	1.7				X	X		X	X	X	X	X			X	X	
E	1.8				X	X		X		X		X	X			X	X
E	2.0		X		X	X			X	X	X	X		X	X	X	X
J	2.0	X	X		X	X	X		X	X	X	X			X	X	

Figures 9-12 are validation comparisons of the VOC profiles of tearless versus tearing onions by SPME-GC-MS where the individual tearless genotypes are represented on the left side of the solid vertical lines and the individual tearing genotypes are represented to the right of the solid vertical lines. These graphs show a clear difference between the two types of onions (tearless vs tearing).

EXAMPLE 6

Minimally Invasive Sweetness, Flavor, or Signs of Ripeness Assessment with Colorimetric Dyes

The methods of Examples 4 and 5 can also provide for an exemplary closed sensor device for combining a colorimetric dye sensing component and a cell damaging component to analyze fruit and vegetables. For example, watermelon VOCs are mainly aldehydes. Any one or more of colorimetric dyes 1-8 and 12-16 from Table 4, above, can be used to detect target aldehyde and/or ketone VOCs in watermelon. The skins or rinds of fruits and vegetables are punctured with a device comprising puncturing members varying in length and thickness based on the thickness and strength of the fruit or vegetable skin or rind. VOCs are analyzed via a colorimetric dye response pattern unique to the particular fruit or vegetable after an exposure

time of at least 2 minutes. There is a very fast reaction between the colorimetric dyes and the fruit or vegetable VOCs in the close-air sampling provided by the device.

- 5 It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

CLAIMS

What is claimed is:

1. A system for detecting one or more volatile organic compounds (VOCs) of interest in a plant sample, the system comprising:
 - 5 (a) one or more colorimetric sensing elements capable of undergoing a color change when contacted by one or more VOCs of interest or a chemical breakdown product thereof; and
 - (b) a cell-damaging component configured to damage at least one plant cell in the plant sample, thereby inducing release of one or more VOCs of interest from the plant
10 sample.
2. The system of claim 1, further comprising a sensor body configured to hold (a) and/or (b) and/or to provide a semi-enclosed or enclosed cavity to receive VOC emissions released from the plant sample when the sensor body is contacted to a
15 surface of the plant sample.
3. The system of claim 1, wherein the plant sample is a fruit, a vegetable, or a part thereof, and wherein the one or more VOCs of interest comprise one or more aldehyde, ketone, or sulfur-containing VOCs, optionally wherein the one or more
20 VOCs of interest are associated with a species of fruit or vegetable and/or with a sensory property of the fruit or vegetable.
4. The system of claim 3, wherein the vegetable is an *Allium* plant, optionally *Allium cepa*, and wherein the one or more VOCs of interest comprise one or more
25 aldehyde, ketone, or sulfur-containing VOCs associated with a sensory property of an *Allium* plant, optionally wherein the sensory property is tearing intensity and/or pungency and/or wherein the one or more VOCs of interest include one or more of the group consisting of propanethial S-oxide, 2-methyl-2-pentenal, 4-methyl-2-pentenal, 4-methyl-3-heptanone, and 3,4-dimethylthiophene.
30
5. The system of any one of claims 1-4, wherein the one or more colorimetric sensing elements comprise one or more colorimetric nanosensors and/or one or more colorimetric dyes.

6. The system of claim 5, wherein the one or more colorimetric sensing elements comprise a plurality of colorimetric sensing elements, optionally at least 5, at least 10, or at least 15 colorimetric sensing elements.

5

7. The system of claim 6, wherein the plurality of colorimetric sensing elements comprise a plurality of colorimetric dyes, optionally wherein each of the plurality of colorimetric dyes is selected from the group consisting of a base indicator, an acid indicator, a solvatochromic dye, an amine-based dye, and a metal salt, optionally wherein the amine-based dye comprises a porphyrin moiety.

10

8. The system of claim 6 or claim 7, wherein the plurality of colorimetric sensing elements are arranged in an array on a surface of a sensor substrate, optionally wherein the sensor substrate is paper or nitrocellulose.

15

9. The system of claim 8, wherein the sensor substrate is configured on a first surface of a sensor body, optionally wherein the sensor body further comprises one or more additional surfaces extending perpendicularly from the first surface, wherein when the sensor body is contacted to a surface of a plant sample while or after the plant sample is contacted with the cell-damaging component, the sensor body forms an enclosed or semi-enclosed cavity to receive VOCs released from the plant sample and wherein said VOC can contact the colorimetric sensing elements of the sensor substrate.

20

10. The system of any one of claims 1-9, wherein the cell-damaging component comprises one or more needles, optionally wherein each or the one or more needles comprises a metal, silica, glass, and/or polymer body having a base and a tip.

25

11. The system of claim 10, wherein the one or more needles are configured to penetrate the plant sample to a depth of at least about 5 millimeters (mm), optionally to a depth of at least about 10 mm, at least about 15 mm, at least about 20 mm, at least about 25 mm, at least about 30 mm, or at least about 35 mm.

30

12. The system of 10 or claim 11, wherein the sensor comprises a sensor body comprising a first surface and one or more additional surfaces extending perpendicularly from the first surface, wherein a sensor substrate comprising an array of a plurality of colorimetric sensing elements is configured on the first surface of the sensor body, and wherein the one or more needles are provided in or on the sensor body and configured where the body of each needle extends perpendicularly from the first surface, wherein when the sensor body is contacted to a plant sample while the plant sample is contacted with the tip of the one or more needles, the sensor body forms enclosed or semi-enclosed cavity to receive VOCs released from the plant sample and where the VOCs can contact the colorimetric sensing elements of the sensor substrate.

13. The system of any one of claims 1-12, wherein the system further comprises a detector for detecting a color change of the one or more colorimetric sensing elements, optionally wherein said detector comprises a camera, an optical microscope, or a smartphone reader, further optionally wherein the system is provided as a handheld or other portable device.

14. A method of detecting one or more volatile organic compounds (VOCs) of interest in a plant, the method comprising:

(i) contacting a plant sample with a cell-damaging component configured to damage at least one plant cell in the plant sample to induce release of one or more VOCs of interest from the plant sample;

(ii) providing one or more colorimetric sensing elements, wherein one or more of the one or more colorimetric sensing agents undergo a color change when one or more VOCs of interest are released by the contacting of (i); and

(iii) detecting a color change associated with one or more of the one or more colorimetric sensing elements, thereby detecting one or more VOCs of interest.

15. The method of claim 14, wherein the plant sample is a sample of a fruit or a vegetable, and wherein the one or more VOCs of interest comprise one or more aldehyde, ketone, or sulfur-containing VOCs, optionally wherein the one or more VOCs of interest are associated with a species of fruit or vegetable and/or with a sensory property of the fruit or vegetable.

16. The method of claim 15, wherein the vegetable is an *Allium* plant, optionally *Allium cepa*, and wherein the one or more VOCs of interest comprise one or more aldehyde, ketone, or sulfur-containing VOCs associated with a sensory property of an *Allium* plant.

17. The method of claim 16, wherein the plant sample is a bulb or leaf of an *Allium* plant, optionally of an *Allium cepa* plant.

18. The method of any one of claims 14-17, wherein the contacting comprises puncturing the surface of the plant sample, optionally wherein the puncturing is performed with one or more needles and/or wherein the puncturing penetrates the plant sample to a depth of at least about 5 mm.

19. The method of claim 18, wherein the one or more colorimetric sensing elements comprises a plurality of colorimetric sensing elements provided in an array.

20. The method of claim 19, wherein the detecting comprises analyzing a color change profile in the array, wherein analyzing the color change profile comprises determining which of the plurality of colorimetric sensing elements in the array change color over a pre-determined time period and/or determining a temporal order of color change of at least two or more colorimetric sensing elements in the array.

21. The method of claim 20, wherein the analyzing further comprises comparing the color change profile in the array to a color change profile provided by performing the method on a control plant sample having one or more known properties.

22. The method of any one of claims 14-21, wherein the detecting is performed visually.

23. The method of any one of claims 14-21, wherein the detecting is performed using a camera, an optical microscope, or a smartphone reader.

24. The method of any one of claims 14-23, wherein the detecting provides information related to the tearing intensity and/or pungency of an *Allium* plant, optionally an *Allium cepa* plant.

5 25. The method of any one of claims 14-24, wherein the method is performed at a site where the plant is grown, stored, or sold.

26. A method of selecting an *Allium* plant having a desired tearing intensity and/or pungency, the method comprising:

10 (i) damaging at least one cell of an *Allium* plant, wherein the damaging induces release of a plurality of volatile organic compounds (VOCs) from the damaged cell of the *Allium* plant but does not impact the viability of the *Allium* plant;

(ii) contacting the plurality of VOCs with an array comprising a plurality of colorimetric sensing elements;

15 (iii) detecting a color change profile in the array, wherein detecting the color change profile comprises determining which of the plurality of colorimetric sensing elements in the array change color over a pre-determined time period and/or determining a temporal order of color change of at least two or more colorimetric sensing elements in the array; and

20 (iv) determining the tearing intensity and/or pungency of the *Allium* plant based on the color change profile of step (iii).

25 27. The method of claim 26, wherein the *Allium* plant is selected from an onion plant, a shallot plant, a leek plant, and a garlic plant, optionally wherein the *Allium* plant is an onion plant.

28. The method of claim 26 or claim 27, wherein the damaging is performed by puncturing the *Allium* plant, optionally wherein the puncturing is performed using one or more needles; by cutting the *Allium* plant; by grinding the *Allium* plant, or by crushing
30 a portion of the *Allium* plant.

29. The method of any one of 26-28, wherein the pre-determined time period is about 2 minutes.

30. The method of any one of claims 26-29, wherein step (iv) comprises comparing the color change profile from (iii) with a color change profile of one or more control *Allium* plants of known tearing intensity and/or pungency.

5 31. The method of any one of claims 26-30, wherein the method comprises selecting a tearless *Allium* plant.

32. A method of developing an *Allium* plant in an *Allium* plant breeding program, the method comprising:

- 10 (i) providing a plurality of *Allium* plants;
- (ii) determining one or more sensory properties of each of the plurality of *Allium* plants based on color change data from one or more colorimetric sensing elements contacted with volatile organic compounds (VOCs) from the *Allium* plant, optionally wherein the color change data comprises a color change profile from an array
- 15 comprising a plurality of colorimetric sensing elements and/or wherein the color change data is provided using a system of one of claims 1-13 or a method of one of claims 14-25; and
- (iii) using the one or more sensory properties determined in (ii) for crossing, selection, or advancement decisions in an *Allium* plant breeding program.

20

33. The method of claim 32, wherein step (iii) comprises: (iii-a) selecting a first *Allium* plant having a first property of interest; (iii-b) selecting a second *Allium* plant having a second property of interest; (iii-c) crossing said first *Allium* plant and said second *Allium* plant to provide a hybrid *Allium* plant; optionally wherein said method

25 further comprises (iii-d) selecting progeny of said hybrid *Allium* plant having one or more properties of interest for further breeding.

34. The method of claim 33, wherein the hybrid *Allium* plant is a tearless *Allium* plant.

30

35. A computer-implemented method of determining a tearing intensity and/or pungency of an *Allium* plant, the method comprising:

- (i) receiving data representing a tearing intensity and/or pungency of an *Allium* plant using one or more colorimetric sensing elements, optionally wherein the data

comprises a color change profile of an array of colorimetric sensing elements contacted with volatile organic compounds (VOCs) from the *Allium* plant and/or wherein said data is provided by using a system of one of claims 1-13 or a method of one of claims 15-20;

5 (ii) comparing the data from (i) to a database comprising data collected from colorimetric sensing elements contacted with VOCs from reference *Allium* plants, wherein the comparing is performed using a computer processor; and

 (iii) generating an output indicating the tearing intensity and/or pungency of the *Allium* plant based on (ii).

10

36. The method of claim 35, wherein the *Allium* plant is an onion plant.

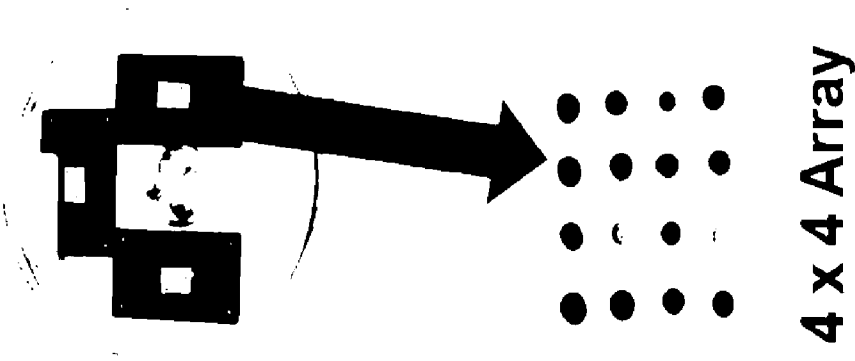


FIG. 2

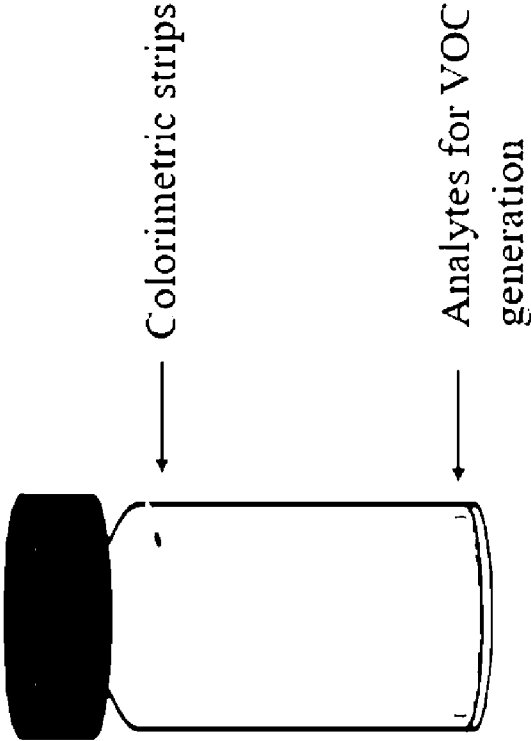


FIG. 1

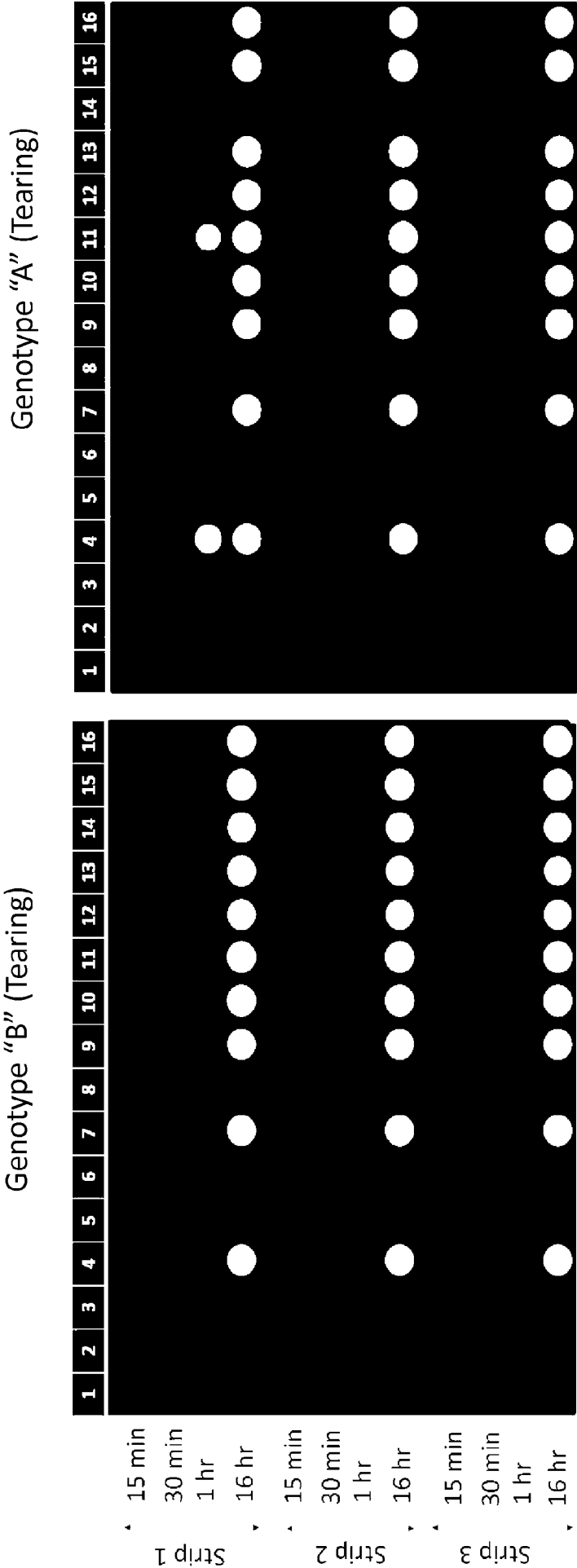


FIG. 3B

FIG. 3A

Genotype “C” (Tearless)

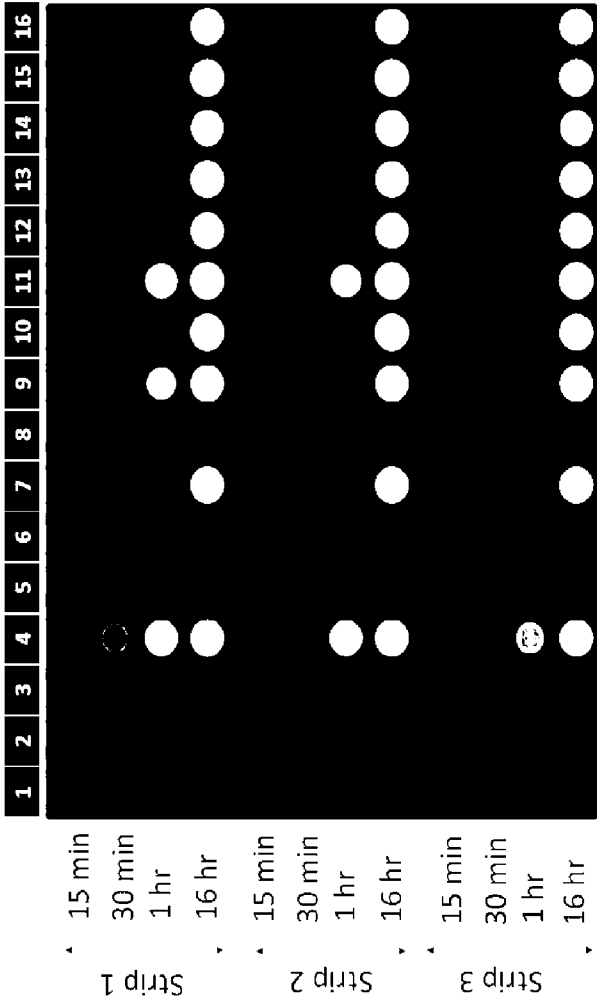


FIG. 3C

Genotype “D” (Tearless)

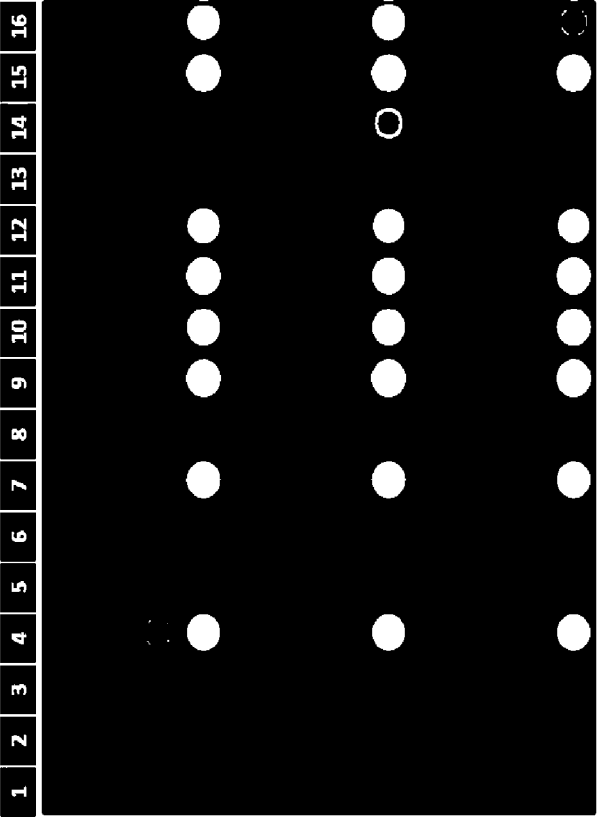


FIG. 3D

A	B	C	D
Type 1		Type 1	
	Dye 15		
	Dye 13		
Dye 13		Dye 13	
		Type 2	
Type 2			
		Dye 12	
		Dye 13	
		Type 3	
Dye 13		Dye 13	
Dye 15			

FIG. 4

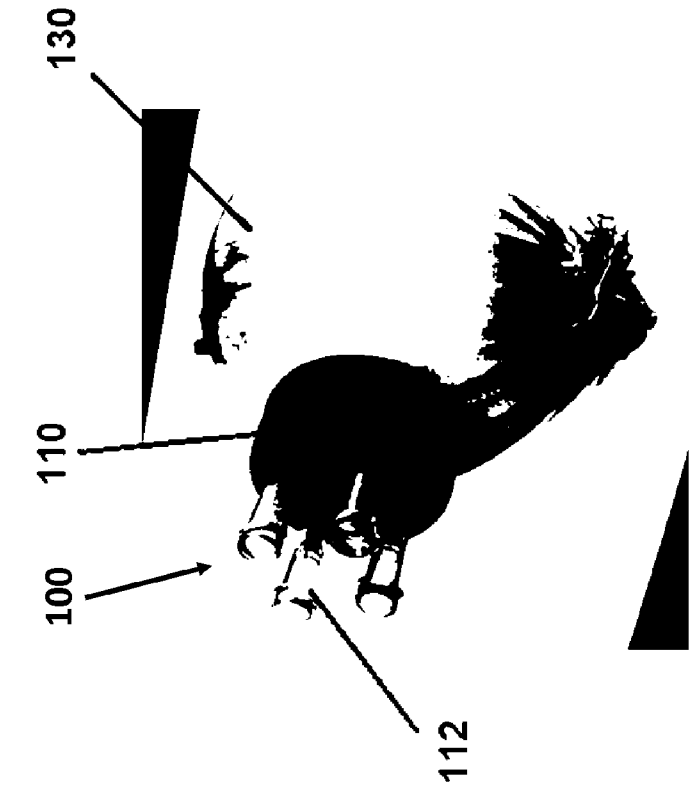


FIG. 5A

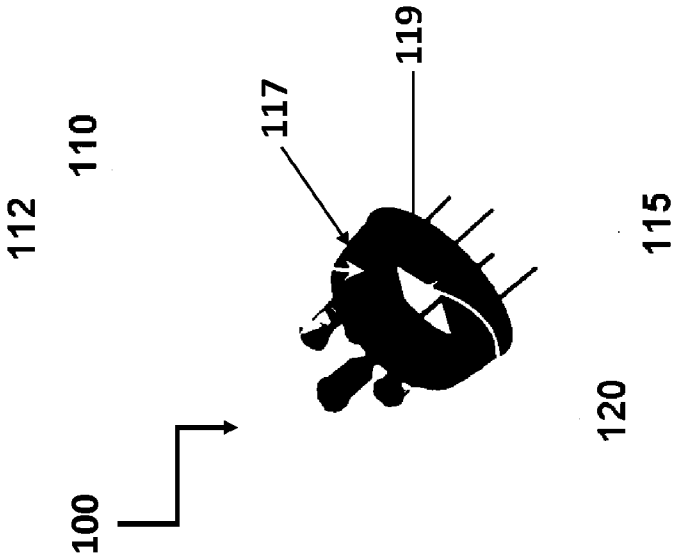


FIG. 5B

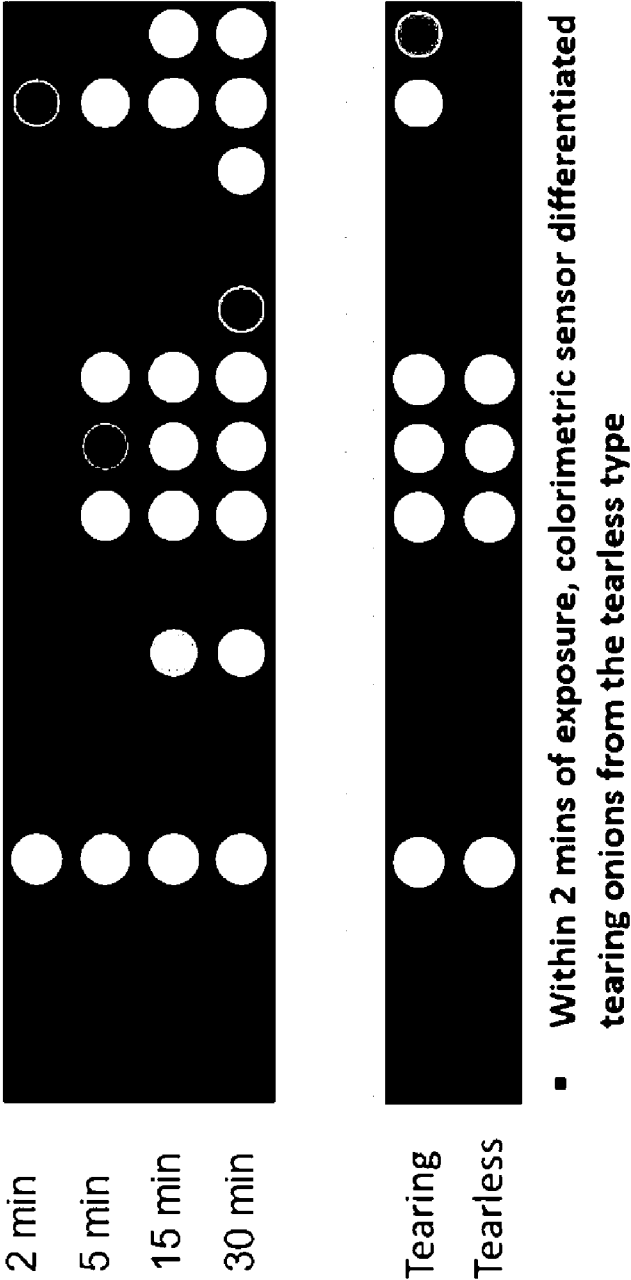


FIG. 6

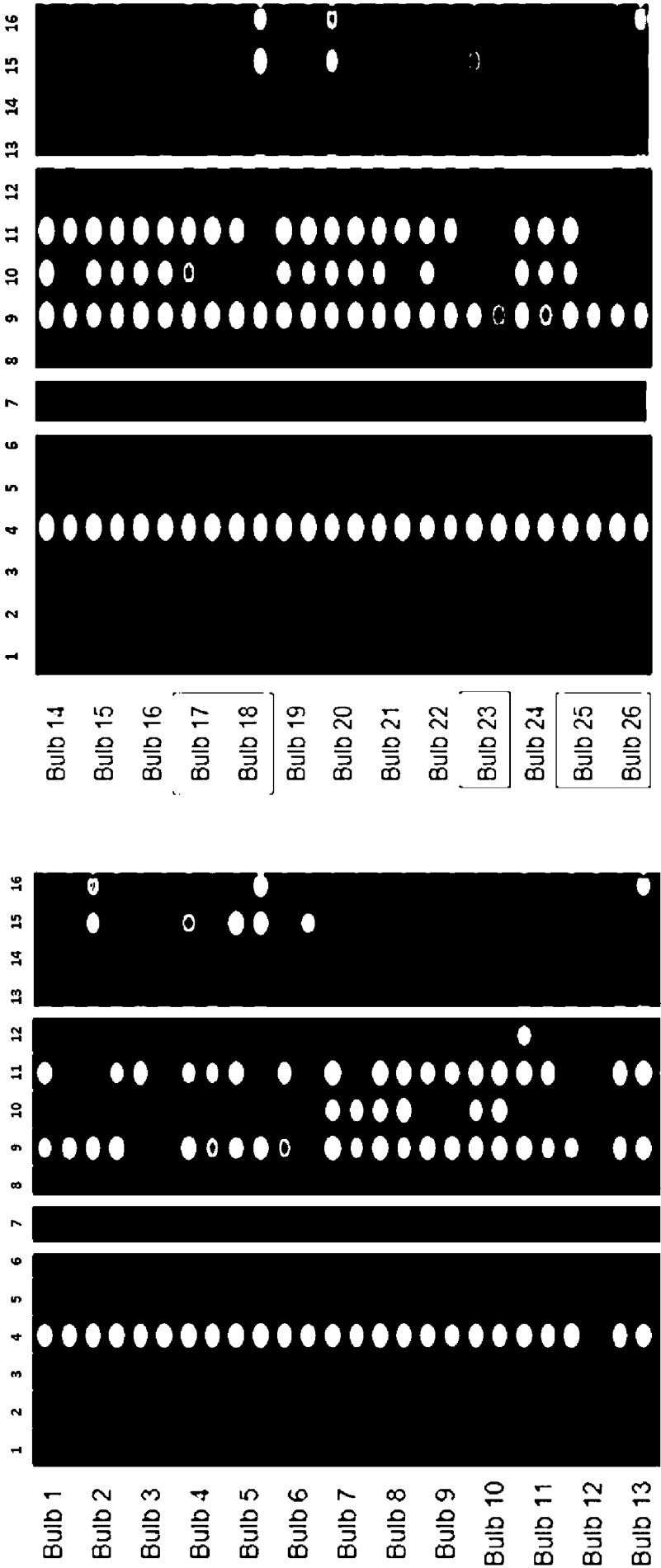


FIG. 7A

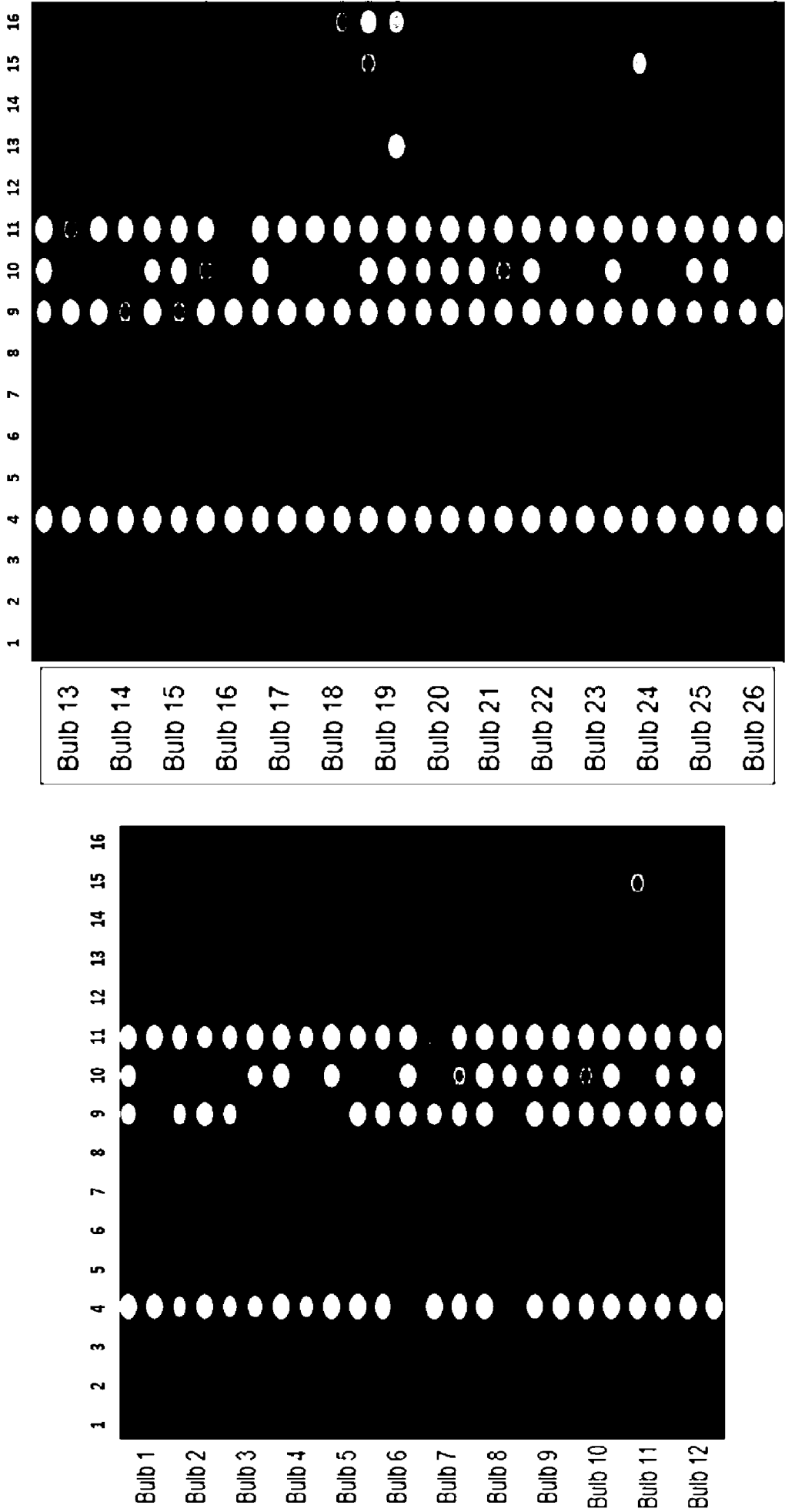


FIG. 7B

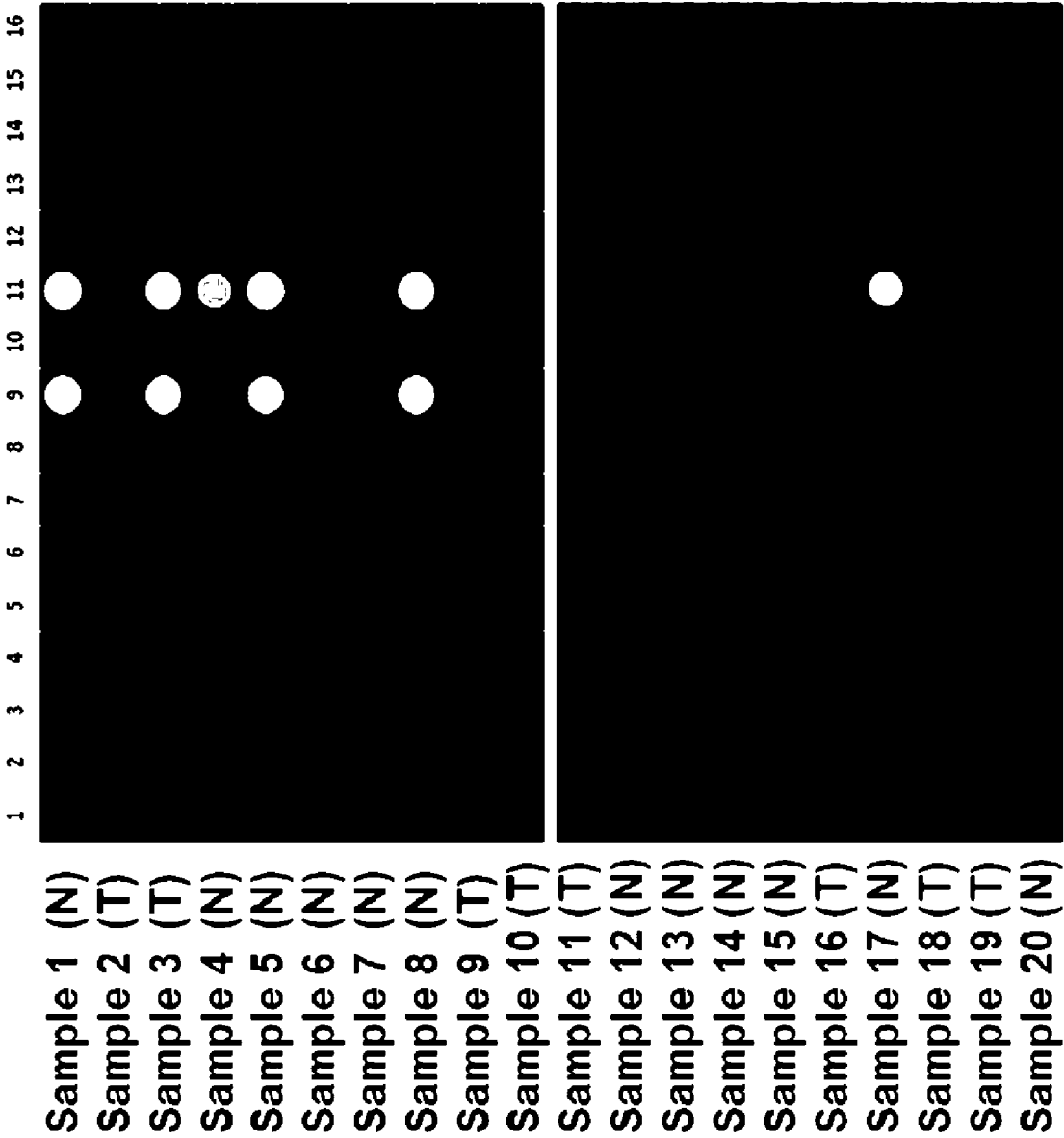


FIG. 8A

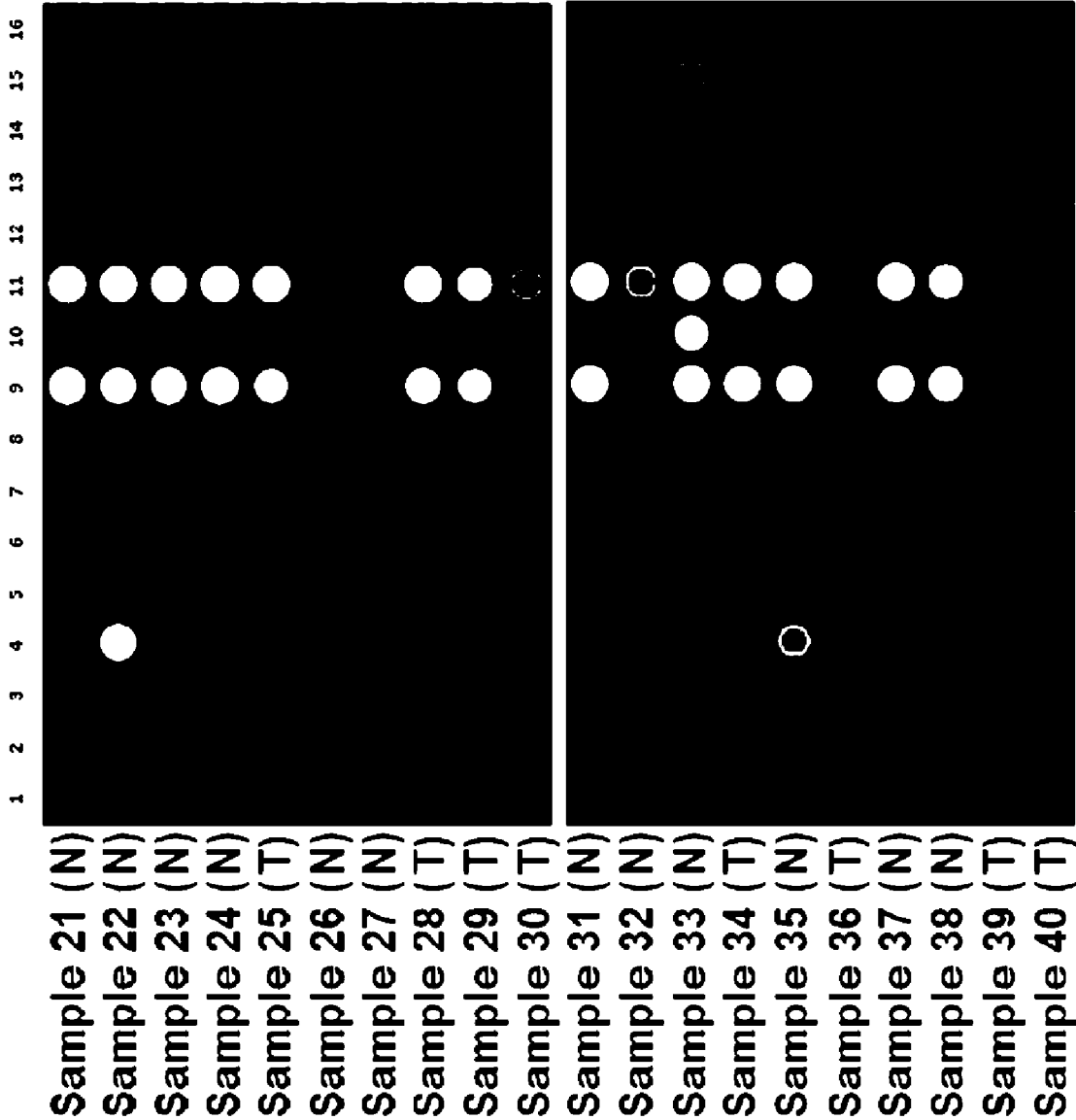


FIG. 8B

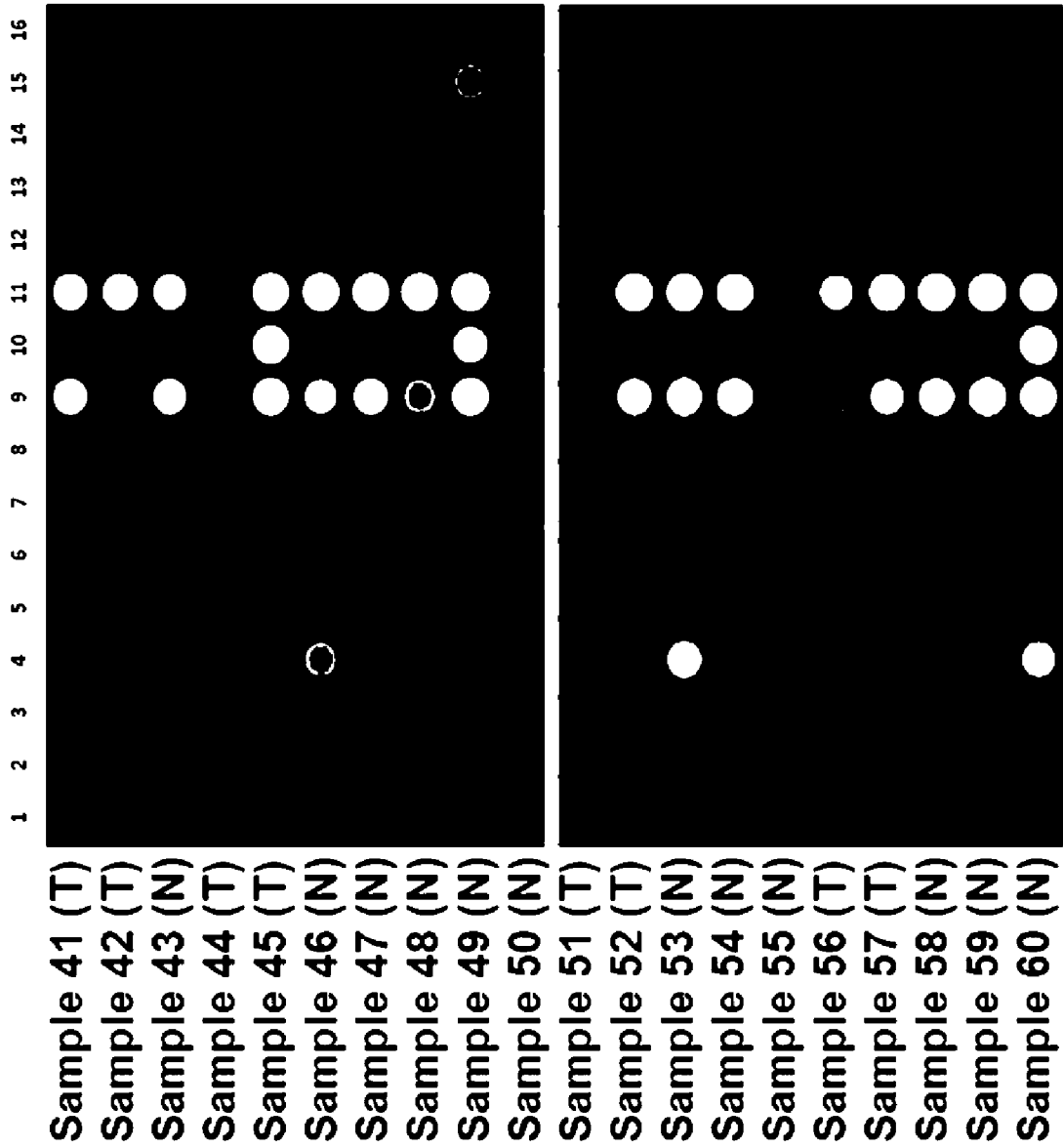


FIG. 8C

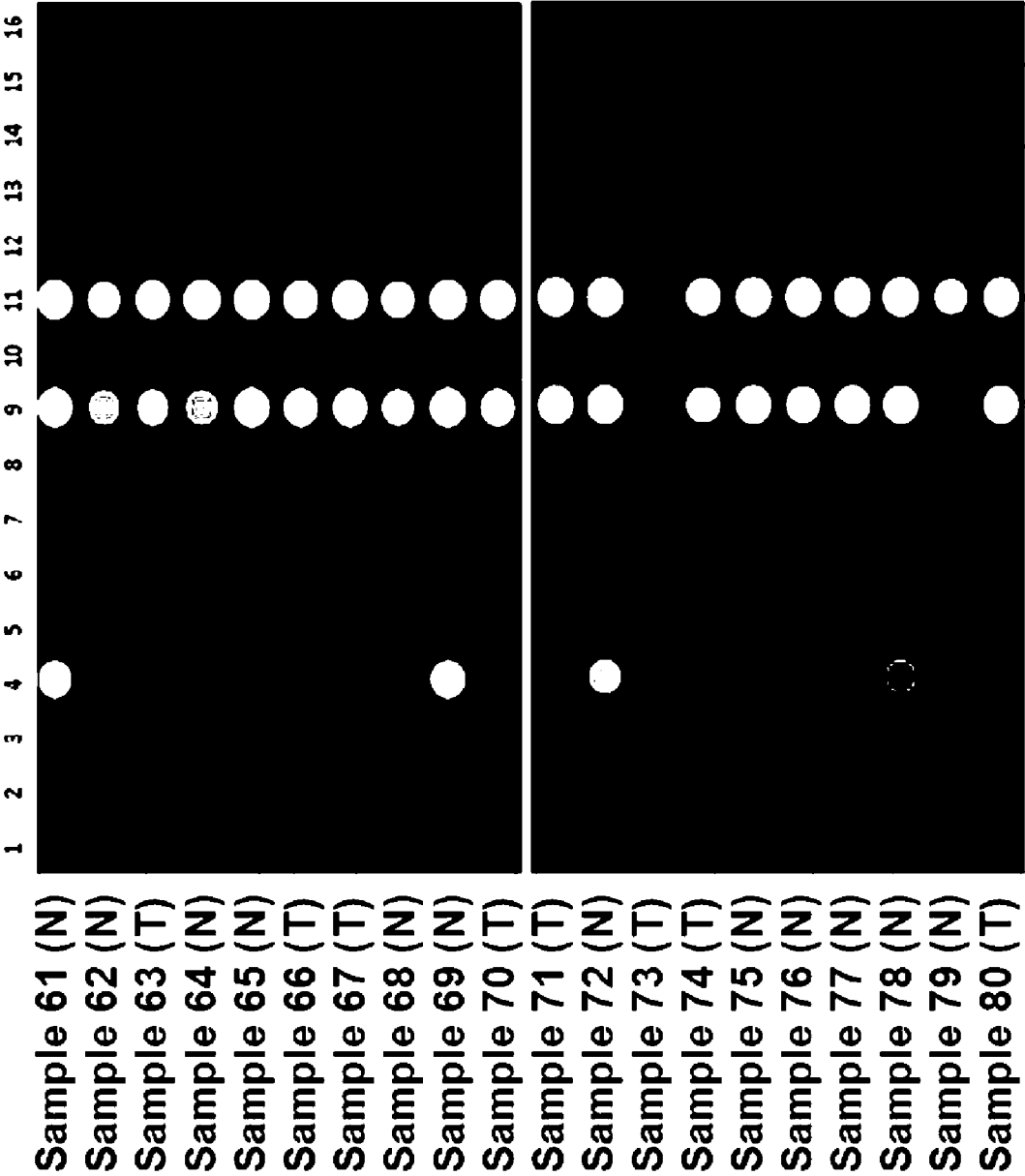


FIG. 8D

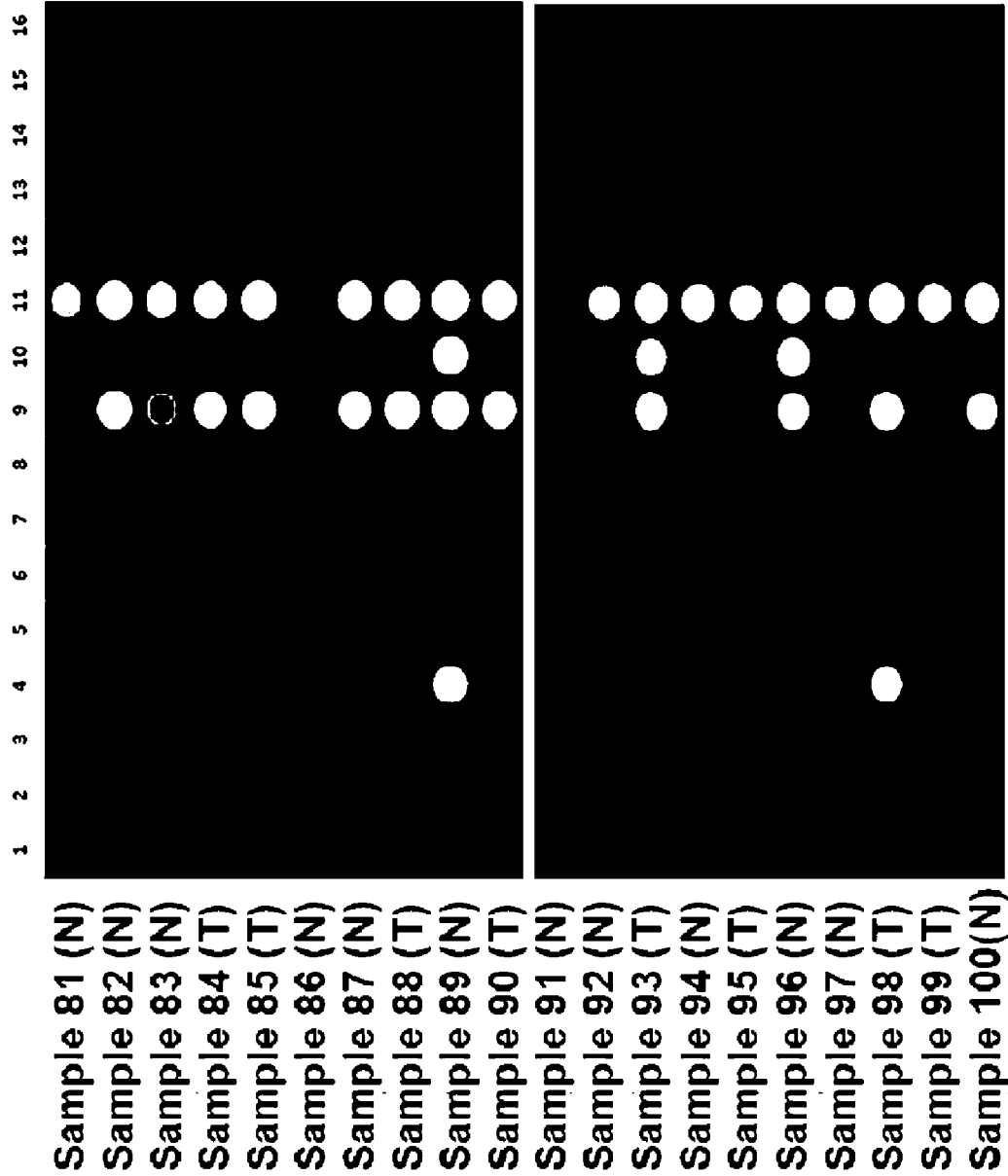


FIG. 8E

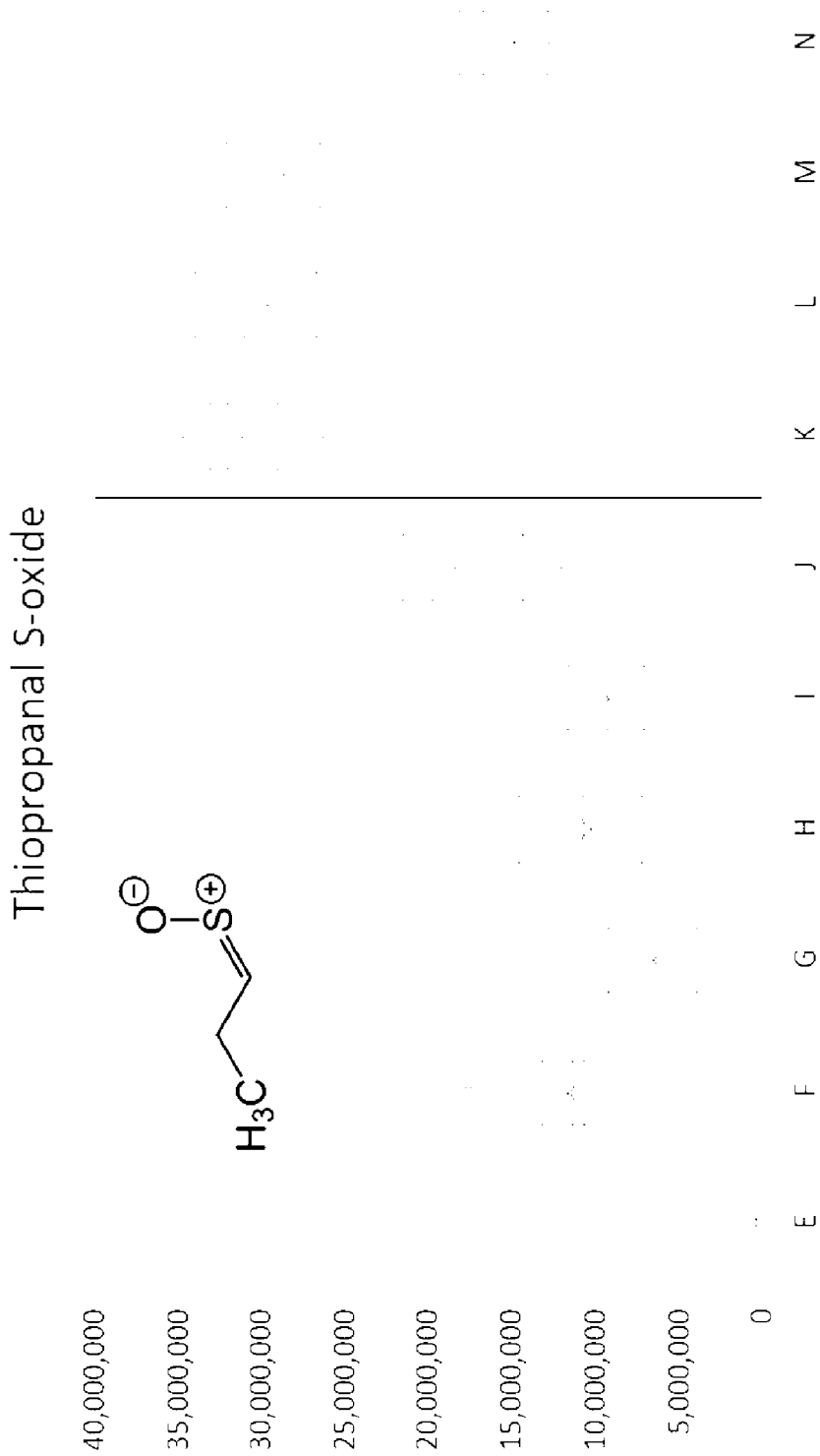


FIG. 9

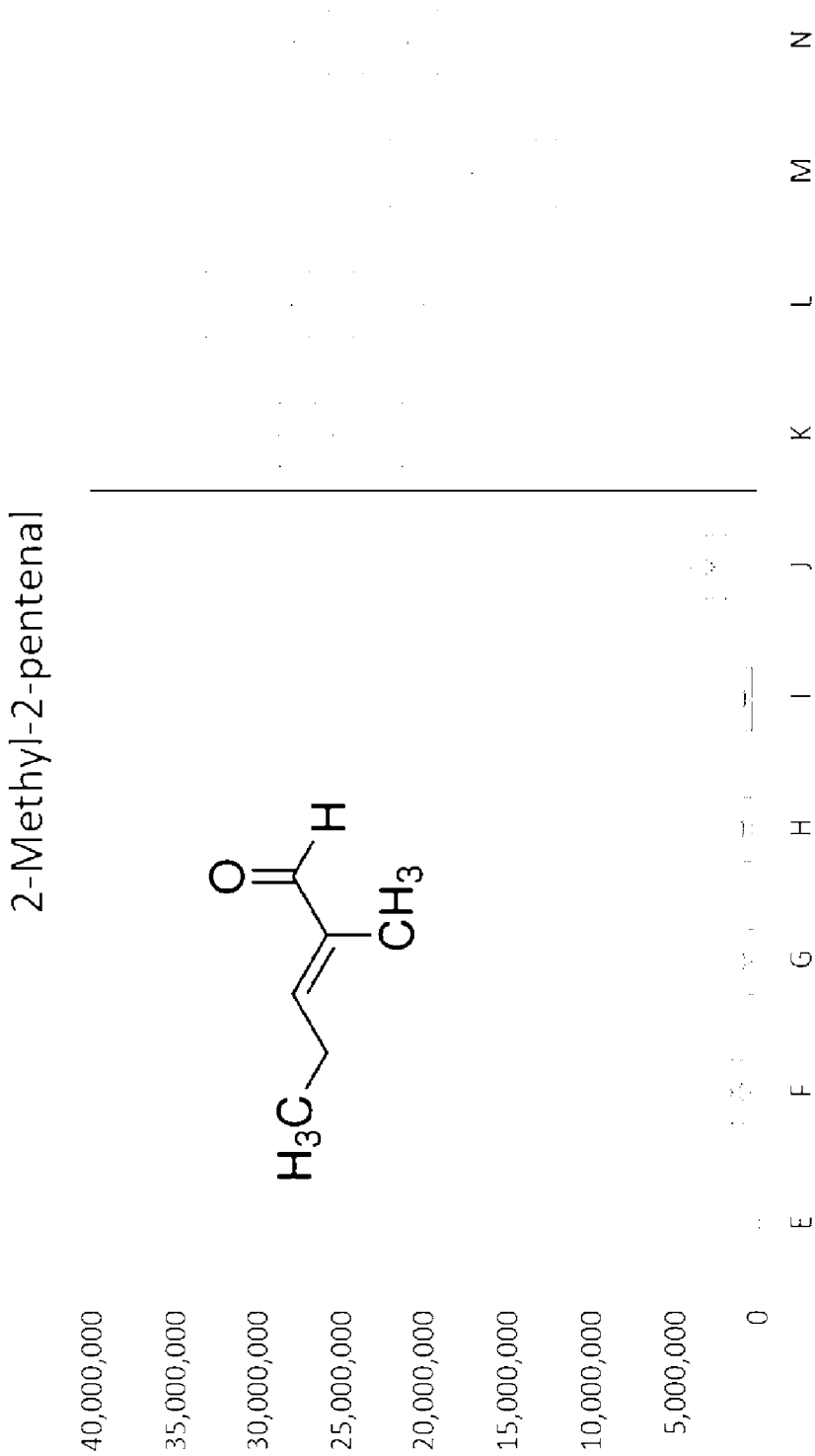


FIG. 10

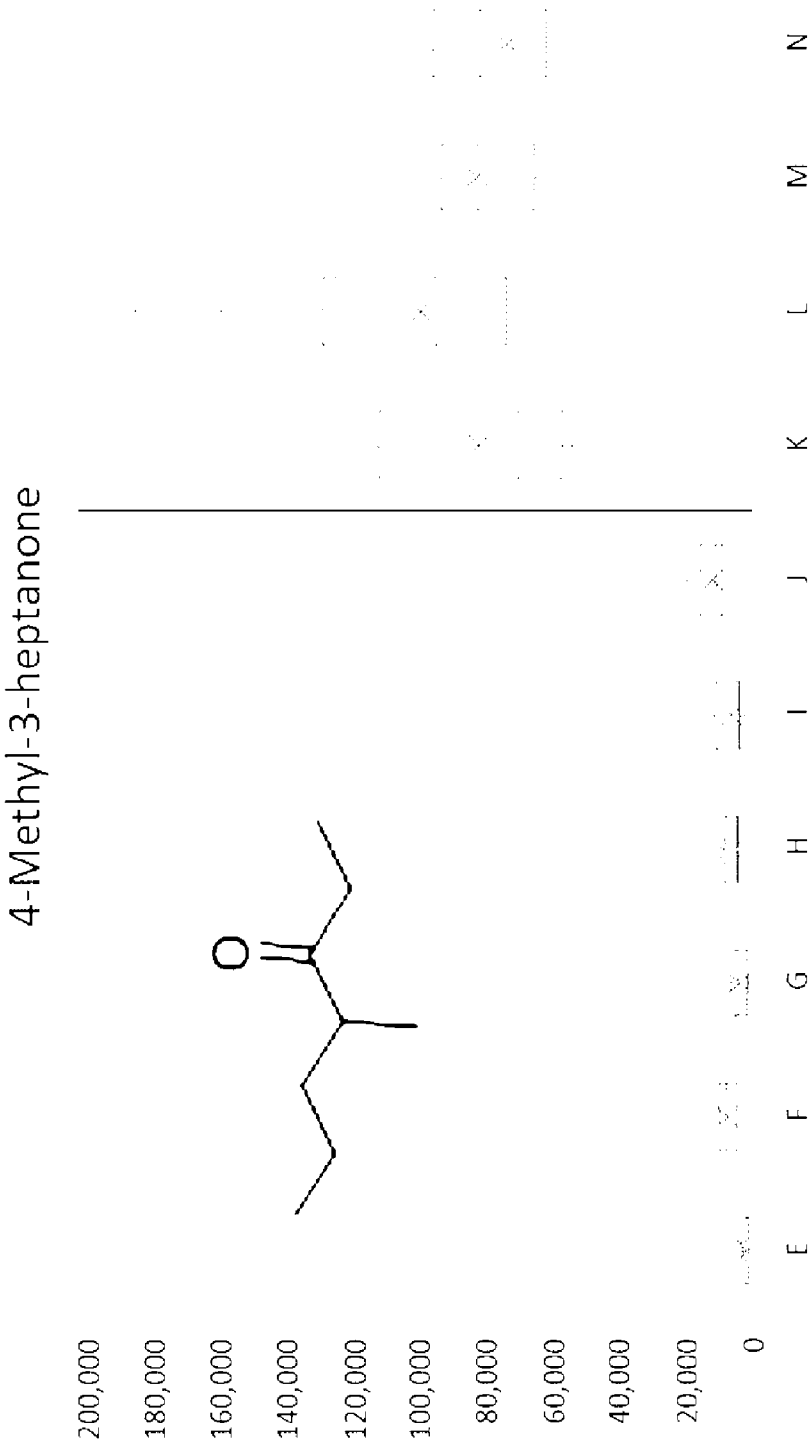


FIG. 11

3,4-Dimethylthiophene

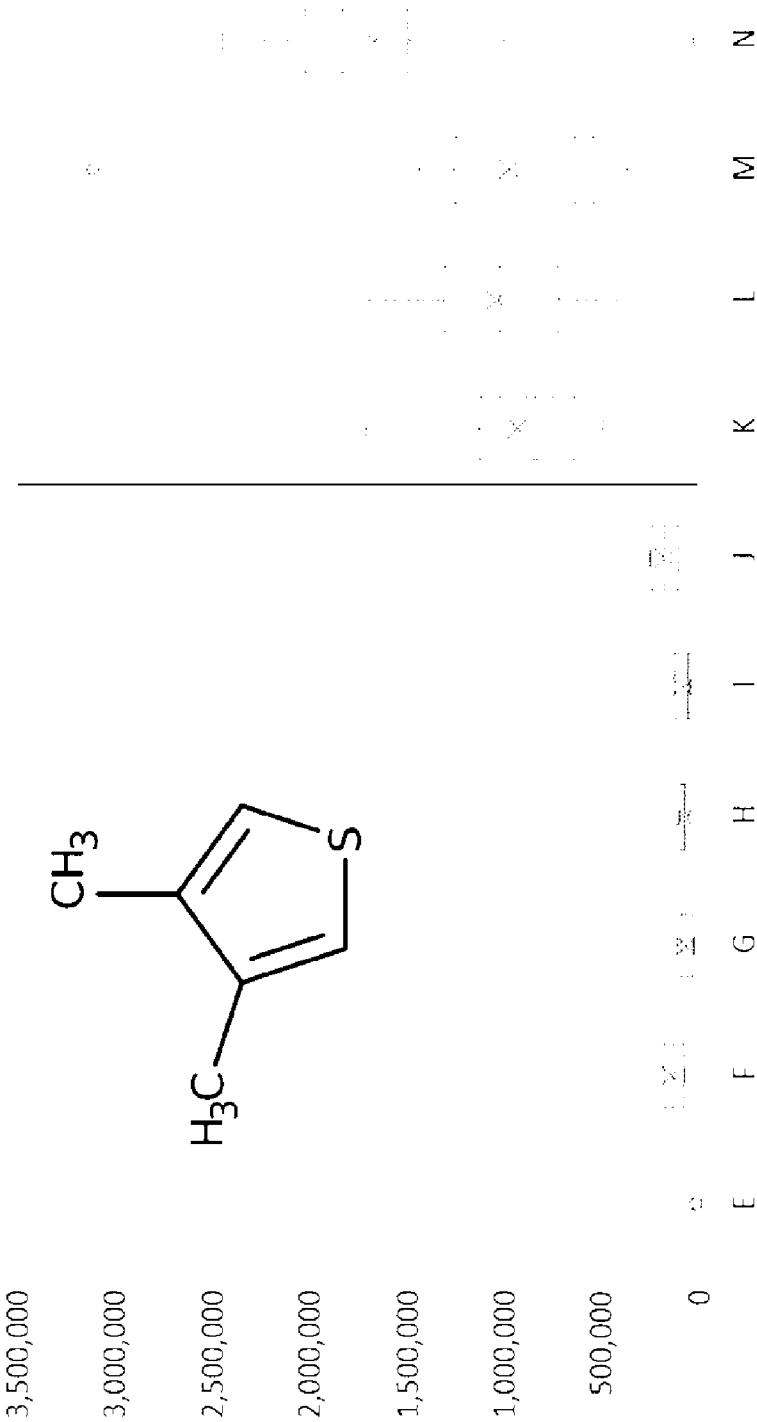


FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/041756

A. CLASSIFICATION OF SUBJECT MATTERIPC: **G01N 21/78** (2023.01); **G01N 1/22** (2023.01)CPC: **G01N21/783; G01N1/22; B82Y15/00; G01N2021/7793; G01N2333/415**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2021/011447 A1 (NORTH CAROLINA STATE UNIVERSITY) 21 January 2021 (21.01.2021) page 10 lines 23-33; page 13 line 30; page 16 lines 7-8; page 18 lines 3-6; page 19 lines 19-24; page 27 lines 16-26; page 28 lines 6-22; page 31 lines 28-30; claim 2; figs. 1, 13A	1-3, 5/1-3, 6/5/1-3, 7/6/5/1-3, 14-15 4, 5/4, 6/5/4, 7/6/5/4, 16-21
Y	KHOT. "IOT Based Food (Onion) Spoilage Detection" 739-746. International Journal Of Multidisciplinary Research In Science, Engineering and Technology (IJMRSET). Web. May 2022; abstract, page 739, last paragraph; page 741, paragraph [8]; DOI: 10.15680/IJMRSET.2022.0505019 abstract, page 739, last paragraph; page 741, paragraph [8];	4, 5/4, 6/5/4, 7/6/5/4, 16-17, 18/16-17, 19/18/16-17, 20/19/18/16-17, 21/20/19/18/16-17

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

- “A” document defining the general state of the art which is not considered to be of particular relevance
- “D” document cited by the applicant in the international application
- “E” earlier application or patent but published on or after the international filing date
- “L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- “O” document referring to an oral disclosure, use, exhibition or other means
- “P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

03 October 2024 (03.10.2024)

Date of mailing of the international search report

13 December 2024 (13.12.2024)

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/041756

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MAJCHRZAK. "Real-Time Volatilomics: A Novel Approach for Analyzing Biological Samples" 1-10. Trends in Plant Science. Web. 2020; page 3, fifth-sixth paragraphs; DOI: 10.1016/j.tplants.2019.12.005 page 3, fifth-sixth paragraphs	18-21

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **8-13, 22-25, 29-36**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: Claims 1-7, 14-21 is directed toward a system and a method for detecting one or more volatile organic compounds.

Group II: Claims 26-28 is directed toward a method of selecting an Allium plant.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a system for detecting one or more VOCs of interest or a chemical breakdown product thereof; contacting a plant sample with a cell-damaging component; wherein one or more of the one or more colorimetric sensing agents undergo a color change when one or more VOCs of interest are released by the contacting; and detecting a color change associated with one or more of the one or more colorimetric sensing elements, thereby detecting one or more VOCs of interest, not present in Group II; the special technical features of Group II include a method of selecting an Allium plant having a desired tearing intensity and/or pungency, the method comprising: (i) damaging at least one cell of an Allium plant, wherein the damaging induces release of a plurality of volatile organic compounds (VOCs) from the damaged cell of the Allium plant but does not impact the viability of the Allium plant; (ii) contacting the plurality of VOCs with an array comprising a plurality of colorimetric sensing elements; (iii) detecting a color change profile in the array, wherein detecting the color change profile comprises determining which of the plurality of colorimetric sensing elements in the array change color over a pre-determined time period and/or determining a temporal order of color change of at least two or more colorimetric sensing elements in the array; and (iv) determining the tearing intensity and/or pungency of the Allium plant based on the color change profile of step (iii), not present in Group I.

Groups I and II share the technical features including: detecting one or more volatile organic compounds (VOCs) of interest in a plant sample; one or more colorimetric sensing elements capable of undergoing a color change when contacted by one or more VOCs of interest; a cell-damaging component configured to damage at least one plant cell in the plant sample, thereby inducing release of one or more VOCs of interest from the plant sample; detecting a color change associated with one or more of the one or more colorimetric sensing elements.

However, these shared technical features are previously disclosed by WO 2021/011447 A1 to NORTH CAROLINA STATE UNIVERSITY (hereinafter 'NCSU'). NCSU discloses detecting one or more volatile organic compounds (VOCs) of interest in a plant sample (a system 100 for detecting one or more volatile organic compounds in a plant sample; page 10 lines 23-24); one or more colorimetric sensing elements capable

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

of undergoing a color change when contacted by one or more VOCs of interest (one or more sensing elements that react with one or more VOC in a gaseous emission, and a detector which detects a signal such as a color change, associated with the reacting of the one or more sensing elements with one or more VOC; a colorimetric VOC sensor array; page 10 lines 29-33, page 13 line 30); a cell-damaging component configured to damage at least one plant cell in the plant sample, thereby inducing release of one or more VOCs of interest from the plant sample (one or more volatile organic compounds in a plant sample, where the gases released by the leaf react with the sensing element; fresh tomato leaves were inoculated by spraying *P. infestans* sporangia suspensions onto the leaf (cell-damaging component), where unique patterns of VOCs related to potential pathogen infection emerged 2 days after inoculation (damaging at least one plant cell, inducing release of VOCs of interest); page 10 lines 22-23, page 16 lines 7-8, page 27 lines 16-18, 25-26); detecting a color change associated with one or more of the one or more colorimetric sensing elements (a detector for detecting a color change associated with the reacting of the one or more sensing elements with one or more VOC in the gaseous emission, where the sensor is a colorimetric sensor array; page 10 lines 30-33, page 19 line 19).

Since none of the special technical features of the Groups I and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the NCSU reference, unity of invention is lacking.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-7, 14-21**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.