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Protocols for Quantifying Transferable Pesticide Residues in Turfgrass Systems

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Abstract:	Plant canopies in established turfgrass systems inherently intercept an appreciable amount of sprayed pesticides, which can be transferred through various routes onto humans. For this reason, transferable pesticide residue experiments are required for registration and re-registration by the US Environmental Protection Agency. Although such experiments are required, limited specificity is required pertaining to experimental approach. Experimental approaches including hand wiping with cotton gloves, modified California roller (moving a roller of known mass over cotton cloth) and soccer ball roll (ball wrapped with sorbent strip) over three treated turfgrass species (creeping bentgrass, hybrid bermudagrass and tall fescue maintained at 0.4, 5 and 9 cm, respectively) are presented. Modified California roller is the most extensively utilized approach to date, and is best suited for use at low mowing heights due to its reproducibility and large sampling area. Soccer ball roll is a less aggressive transfer approach; however, it mimics a very common occurrence in the most popular international sport, and has many implications for nondietary pesticide exposure from hand-to-mouth contact. Additionally, this approach may be adjusted for other athletic activities with limited modification. Hand wiping is the best approach to transfer pesticides at higher mowing heights, as roller-based approaches can lay blades over; however, it is more subjective due to more variable sampling pressure. Utility of these methods across turfgrass species is presented, and additional considerations to conduct transferable pesticide residue research in turfgrass systems are discussed.
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TITLE:
Protocols for Quantifying Transferable Pesticide Residues in Turfgrass Systems

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SHORT ABSTRACT:
Transferable pesticide residue experiments in turfgrass systems are integral components of human risk exposure assessments. Experimental approaches to measure transferable residues should be adjusted to the human interaction of interest and turfgrass system dynamics. Three transferable pesticide residue protocols are presented and the suitability across three turfgrass systems is discussed.

LONG ABSTRACT:
Plant canopies in established turfgrass systems can intercept an appreciable amount of sprayed pesticides, which can be transferred through various routes onto humans. For this reason, transferable pesticide residue experiments are required for registration and re-registration by the United States Environmental Protection Agency (USEPA). Although such experiments are required, limited specificity is required pertaining to experimental approach. Experimental approaches used to assess pesticide transfer to humans including hand wiping with cotton gloves, modified California roller (moving a roller of known mass over cotton cloth) and soccer ball roll (ball wrapped with sorbent strip) over three treated turfgrass species (creeping bentgrass, hybrid bermudagrass and tall fescue maintained at 0.4, 5 and 9 cm, respectively) are presented. The

modified California roller is the most extensively utilized approach to date, and is best suited for use at low mowing heights due to its reproducibility and large sampling area. The soccer ball roll is a less aggressive transfer approach; however, it mimics a very common occurrence in the most popular international sport, and has many implications for nondietary pesticide exposure from hand-to-mouth contact. Additionally, this approach may be adjusted for other athletic activities with limited modification. Hand wiping is the best approach to transfer pesticides at higher mowing heights, as roller-based approaches can lay blades over; however, it is more subjective due to more variable sampling pressure. Utility of these methods across turfgrass species is presented, and additional considerations to conduct transferable pesticide residue research in turfgrass systems are discussed.

INTRODUCTION:

Turfgrasses are grown on over 16.3 million ha in the contiguous United States (US) – exceeding the combined area of irrigated grain corn [(*Zea mays* L.) 2.5 million ha], soybeans [(*Glycine max* L.) 2.1 million ha] and cotton [(*Gossypium hirsutum* L.) 0.9 million ha] – and are utilized by the public with land uses including athletic fields, commercial/residential lawns, golf courses and parks[1,2]. They provide many positive societal attributes including dust control, heat dissipation, recreational surfaces and soil stabilization. However, pest encroachment may occur which requires the use of pesticide(s) to maintain the turf to an acceptable level[3].

Plant canopies in established turfgrass systems intercept sprayed pesticides. Human-pesticide exposure is possible via various routes, including transferring from treated vegetation directly onto humans, as well as through indirect routes such as transfer onto maintenance equipment, pets and recreational items[4,5]. To address such concerns, human pesticide exposure risk assessments must be conducted prior to pesticide registration and re-registration in the U.S. to estimate the nature and probability of adverse health effects following exposure to contaminated environmental media[6]. Within the occupational and residential exposure test guidelines currently employed by the USEPA, foliar transferable residue dissipation tests (OPPTS 875.2100) are conducted to quantify pesticide residues on treated surfaces that can be transferred through various processes onto human skin/clothing or inhaled[7,8].

Previous research efforts have compared several foliar transferable pesticide residue methods including a California roller (moving a roller of known mass over cotton cloth), drag sled (pulling a solid object of known mass with a piece of cloth attached to it), polyurethane foam roller (moving a roller of known mass covered with polyurethane foam) and shoe shuffling (attaching cotton cheesecloth to shoes), which are all conducted in a known area of pesticide-treated turfgrass[9-12]. Of the aforementioned methods, California roller-based approaches provide the most repeatable approach to quantify foliar transferable pesticide residues; however, comparably more aggressive approaches such as shoe shuffling can transfer more pesticide residue, which also has utility in risk assessments. Hand wiping methods provide a comparatively enhanced ability to contact unique treated turfgrass vegetation surfaces. This method provides more applicable data for nondietary pesticide ingestion, as hand-to-mouth contact is a common process associated with this exposure route.

Turfgrass canopy dynamics vary widely between both species and use sites. Species commonly vary in growth habit and season, as well as blade texture and density, which affect pesticide spray interception and physiological processes[3,13]. Management inputs can vary widely between use sites, and within a use site based on site-specific expectations. For example, bermudagrass (*Cynodon* spp.) is utilized in adapted climates as a golf course putting green surface which are typically irrigated and mown $\geq$ 5 times per week (clippings collected) at 0.3 to 0.4 cm, as well as a nonirrigated utility ground cover which may be mown $\leq$ 1 time week^{-1} (clippings returned) at 1.5 to 6.3 cm. Previous research has shown transferable foliar pesticide residues can vary between species within a use site, and is affected by irrigation and mowing practices[14,15]. Ultimately, variability between turfgrass systems inhibits the implementation of a universal method to quantify transferable foliar pesticide residues. Therefore, method selection to optimize human risk assessments should encompass pesticide-, process-, site- and species-specific criteria. The objective of this study was to characterize various methods used to quantify transferable foliar pesticide residues, and highlight conditions that should be considered when selecting a method for a given experiment.

PROTOCOL:

1. Field plot identification and establishment

1.1) Identify research areas representative of the turfgrass system of choice. Specifically consider appropriate turfgrass species and soil type, as well as management practices including fertility, irrigation and mowing height.

1.2) Conduct preliminary residue analyses of foliage and soil matrices to ensure pesticide(s) of interest are not detectable.

1.3) Lay out plots to appropriate dimensions for site and sample collection methods, and incorporate 0.5-1.0 m alleys on plot borders to allow for personnel foot traffic.

Note: Plot dimensions can vary based on site-specific conditions, but should be a minimum of 3.3 m^2. This will allow for a 3.7 m x 0.5 m ball travel pathway, as well as an equivalent adjacent area for foot-traffic and vegetation sampling.

2. Trial initiation

2.1) Twenty-four hours prior to initiation mow turfgrass to the species-system specific height and collect clippings. Remove all remaining debris with a blower and irrigate to soil saturation.

Note: Irrigating at this time should allow for optimal moisture (i.e. field capacity) at pesticide application.

2.2) Select pesticide application rate at the currently registered maximum labeled allowances for the site of interest. The application rate is product-specific and determined by the USEPA, and is stated by law on all pesticide container labels.

2.2.1) Select the current minimum labeled spray application carrier volume to maximize foliar-spray retention.

2.3) At trial initiation, apply pesticide spray treatments to plots when climatic conditions favor retention on treated vegetation.

Note: The proper personal protective equipment must be worn by applicators and nonapplicators near the test area during the treatment period. Always keep nonapplicator personnel upwind of spray application. Climatic conditions favoring retention on treated vegetation include canopy moisture (dry at application), wind speed ($\leq$ 11 km/h) and forecasted precipitation

2.3.1) Cover plots during irrigation/precipitation through at least four sampling events.

Note: Plots are covered to prevent pesticide wash from treated vegetation into turfgrass thatch and/or the soil surface, making it less dislodgeable and underestimating exposure risk. Covers can vary by material and design, but should be constructed of a nonporous material to keep plots dry and designed such that treated vegetation is not contacted (ex. do not lay tarps directly onto plots), which may compromise subsequent pesticide transfer sampling.

2.4) Collect hourly climatic data including air temperature, dew point, relative humidity, time of sunrise, wind direction and wind speed throughout the trial period to aid with data interpretation.

3. Quality control samples

3.1) Collect one sample (50 to 100 mL) from each pesticide container used in the research to ensure pesticide-product loading rates are correct. Record product name and lot number of each container.

3.2) Collect a sample of each pesticide spray tank mix applied by collecting output from each spray nozzle to make one composite sample (50 to 100 mL) per tank to ensure correct spray solution mixing procedures.

3.3) Produce spray application controls as described by Welsh *et al.*[12] to ensure the appropriate amount of pesticide is applied to plots.

3.4) Produce field fortification and blank samples as described by Welsh *et al.*[12] at each sampling event to quantify dissipation during the sample collection and storage processes, and to identify potential sources of contamination.

Note: Fortification sample concentrations and solvents can vary based on compound- and analytical-specific considerations.

4. Sample collection considerations

4.1) Collect samples immediately after an application, and again once the pesticide has dried on the leaf surfaces. Depending on environmental conditions, this is typically 1 to 3 h after application.

4.2) Following the day of application, collect samples at, or just after sunrise, when maximum turfgrass canopy moisture is typically present[14].

4.2.1) Determine sample collection timings after the day of application based on specific pesticide physicochemical properties, with sampling intervals decreasing as pesticide persistence decreases. Schedule sample collection timings such that at least two consecutive nondetections occur by experiment completion.

Note: Example from previous research quantifying transferable 2,4-dimethylamine salt (2,4-D; field half-life = 6.2 d): 1) 0 d after treatment (DAT) – 0 h after spraying; 2) 0 DAT – 1 h after spraying; 3) 1 DAT; 4) 2 DAT; 5) 3 DAT; 6) 6 DAT; 7) 12 DAT; 8) 24 DAT[14].

5. Transferable residue sample collection

Note: Nitrile gloves are worn throughout all sample collection procedures, and should be replaced as often as needed to prevent contamination.

5.1) Hand wipe

Note: Requires one person.

5.1.1) Calibrate hand wiping pressure to 2 kPa.

5.1.1.1) Calculate wiping force by securing a turfgrass core collected at soil field capacity to a digital scale and pressing to provide 150 to 200 g (tare prior to pressing).

Commented [A1]:

[Place Figure 1 here]

5.1.1.2) Quantify area of front- and backhand used for turfgrass sampling. Primarily use fingers to contact unique turfgrass blades while limiting them from laying over.

5.1.1.2.1) Prepare a 10% v/v water-based solution of green food coloring and a 1% v/v nonionic surfactant, and spray on a nonporous surface to create a thin film.

Note: The nonionic surfactant is used to increase solution coverage over the nonporous surface by reducing surface tension.

5.1.1.2.2) With a cotton glove on, press hand on the nonporous surface (Figure 1).

5.1.1.2.3) Remove the glove and quantify area of hand in contact with the nonporous surface by taking a digital photo over a known area of each glove, and use ImageJ[16] to select for green pixels similarly to Campillo *et al.*[17]. This will provide a percent of the total image in green pixels, which is used to calculate area.

5.1.1.3) Combine wiping force mass with wiping area to calculate hand pressure.

5.1.2) Calibrate hand wiping speed to 1.2 km/h by marking a 0.3 m distance on the ground. Adjust the hand speed such that wiping this distance takes 1 s.

5.1.3) Mark sampling area (420 cm^2) by placing a circular bucket (23 cm diameter) upside down where the open end is facing downward. Apply light pressure to the center of the bucket and with opposite hand paint around the outer edge of the bucket (using turf marking paint).

5.1.4) With a cotton glove over a nitrile glove on the dominant hand, wipe over the sampling area for 30 s. Alternate front- and backhand surfaces, while adjusting directions perpendicularly (Figure 2).

[Place Figure 2 here]

5.1.4.1) Repeat step 5.1.4 with a new cotton glove.

Note: Variation in gloves required per sample can vary based on the pesticide of interest, turfgrass system and sampling area. This should be determined prior to experiment initiation via preliminary efforts collecting four to six gloves per sampling area immediately after application, as well as the following morning when canopy moisture is present to identify the number of gloves to transfer maximum pesticide residue.

5.1.5) Following sample collection, store gloves in a glass jar temporarily on ice or dry ice in the field, and transfer to a freezer within 3 h following sample collection completion.

5.2) Modified California roller
Note: Requires three people. Apparatus building specifications found at Fuller et al.[9]

5.2.1) Use a roller (roller: 14.5 kg, 10 cm diameter by 61 cm length covered with 1.2 cm polyurethane foam; handle: 1.2 m length) and a frame (62 cm width by 91 cm length) that a cotton sheet (> 200 thread count; 70 cm width by 99 cm length) is secured to.

5.2.2) Have Persons B and C secure cotton sheet to frame with clamps and secure to sampling area with metal nails.

Note: Cut cotton sheets prior to a sample collection event to reduce time for a given collection.

5.2.3) Have Person A and B secure plastic (6 mm) around the roller to prevent contamination across samples.

5.2.4) Without adding down pressure, have Person A roll the roller over the cotton cloth five times (one down and back roll equals one time). Roll speed should target 1.6 ± 0.2 km/h.

5.2.5) Have Person B remove the frame from the ground and take to a nearby sampling table.

5.2.6) While Person B is holding the frame, have Person C visually inspect the cloth and remove grass/soil debris with tweezers.

5.2.7) Have Person A remove the cotton cloth, fold it such that the side in contact with turfgrass is folded together and store in glass sample jar.

5.2.7.1) Store the glass jar temporarily in a cooler on ice or dry ice in the field, and transfer to a freezer within 3 h following sample collection completion.

5.2.8) Following sample collection, discard the plastic surrounding the roller, clean the frame, sampling table and tweezers with 1:1 ammonia:water, rinse with water and dry prior to repeating the process.

5.3) Soccer ball roll
Note: Requires one person.

5.3.1) Use a soccer ball roller frame constructed of polyvinyl chloride (PVC) pipe (schedule 40; 5 cm inner diam).

[Place Figure 3 here]

5.3.1.1) Secure the handle (122 cm length; Figure 3 – A) to a slip tee coupler (Figure 3 – B). This is the only junction not secured with PVC glue to make for easier storage.

5.3.1.2) Lay the roller on a flat surface (aids with alignment) and secure both sides of the tee coupler to slip 90° elbow fittings (Figure 3 – C) with 7 cm pipe length (Figure 3 – D).

5.3.1.3) Insert 24 cm pipe lengths (Figure 3 – E) to the open end of the elbow fittings

5.3.1.4) Drill lag bolts (0.6 cm diam by 7.5 cm length; Figure 3 – F) through the end of the pipe perpendicular to the handle and parallel with the flat surface.

5.3.1.4.1) Set distance between lag bolts to approximately 18.4 cm (can vary across brands/quality of soccer balls used).

5.3.1.4.2) File/grind sharp point off lag bolt ends with a hand file/grinder so that soccer balls are not punctured when rolling (Figure 3 – G).

5.3.2) Release soccer ball air pressure to 24 kPa. Although this will underinflate the ball when loose from the PVC roller, it will increase pressure to approximately 42 kPa when the ball volume is reduced after securing to the roller.

5.3.3) Mount the soccer ball onto the roller by touching the ball endpoints (maximum diameter) to lag bolts.

Note: To reduce overall sampling time and potential contamination, have one soccer ball per sample at a given collection timing.

5.3.3.1) Spin ball by hand to ensure the ball roll is symmetrical.

5.3.3.2) Press lightly on top of the mounted soccer ball to ensure it is adequately secured.

5.3.4) Double-wrap a sorbent strip (5 cm width by 132 cm length) around the circumference of the ball that aligns with the PVC roller handle, which creates constant turfgrass-sorbent strip contact when rolling (Figure 3 – H).

5.3.4.1) Secure the sorbent strip to the ball with adhesive tape strips (2.5 cm by 2.5 cm).

Note: Cut sorbent strips prior to a sample collection event to reduce time for a given collection.

5.3.5) Roll the ball at a constant speed (1.6 ± 0.2 km/h) over a 3.7 m distance of unique treated turfgrass. Depending on plot dimensions, this distance may require side-by-side rolls to complete. When doing so, take precaution to not contact vegetation that has yet to be sampled.

Note: The 3.7 m ball roll distance may need to be adjusted based on the pesticide of interest and turfgrass system. This should be determined prior to experiment initiation via preliminary efforts collecting samples of varying distances immediately after application, as well as the following morning when canopy moisture is present to identify the optimal distance to transfer maximum pesticide residue.

5.3.6) Following the ball roll, remove the sorbent strip and fold it such that the side in contact with turfgrass is folded together and store in a glass jar.

5.3.6.1) Store the glass jar temporarily in a cooler on ice or dry ice in the field, and transfer to a freezer within 3 h following sample collection completion.

6. Turfgrass vegetation collection

6.1) Remove the plunger inside of the cup cutter to keep sampling equipment from contacting treated foliage (Figure 4 – A).

[Place Figure 4 here]

6.2) Harvest a turfgrass core of representative quality.

6.2.1) Use the cup cutter to outline the core and cut to 5-7.5 cm soil depth to aid with removal.

6.2.2) Cut with a knife (blade length: 10-15 cm) at a diagonal angle outside the edge of the core to remove the core from the ground. Pay special attention to prevent hand/knife contact with treated aboveground vegetation.

6.2.3) Level the bottom soil surface with scissors so that it will sit flat in a sample container. Soil depth should allow enough space such that turfgrass vegetation does not contact the sample container lid.

Note: When weather permits, the golf course cup cutter step (6.2.1 in protocol) is typically completed in the afternoon when foliage is dry before sample collection the following morning.

REPRESENTATIVE RESULTS:

Building on previous research efforts comparing transferable pesticide residue methods within a single turfgrass system, and turfgrass systems within a single transferable pesticide residue method, a field study (initiated May 24, 2016 in Raleigh, North Carolina, USA) was conducted to compare methods across turfgrass systems. In short, 2,4-D, a broadleaf herbicide used commonly in turfgrass systems, transfer from three turfgrass species (creeping bentgrass, *Agrostis stolonifera* L.; hybrid bermudagrass, *Cynodon dactylon* L. x *C. transvaalensis* Burtt-Davey; tall fescue, *Lolium arundinaceum* [Schreb.] S.J. Darbyshire) via three methods (hand wipe, modified California roller or soccer ball roll) was quantified immediately after application and a 1 h drying period, as well as 1 and 3 DAT. Creeping bentgrass mowing height simulated a golf course putting green at 0.4 cm, while hybrid bermudagrass and tall fescue were maintained at 5 and 9 cm, respectively, which is representative of commercial/residential lawns and parks. Research areas were not mown and covered during rainfall following broadcast 2,4-D spray application (1 kg active ingredient ha^{-1}).

Compared to previous reports evaluating transferable 2,4-D from treated turfgrass, data from the presented research suggest conditions were favorable for transfer across all methods. Averaged over methods, 2,4-D transfers immediately after application ranked creeping bentgrass (21% of applied) > hybrid bermudagrass (16.4%) > tall fescue (15.1%), which aligns with canopy density trends across systems (Table 1; Figure 5). Averaged over turfgrasses, 2,4-D transfers immediately after application ranked hand wipe (21.2% of applied) > modified California roller (16.8%) > soccer ball roll (14.4%), which agrees with previous efforts showing hand/shoe sampling can transfer more pesticide residue compared to other transfer techniques including drag- and roller-based methods[10,11]. A 1 h drying period resulted in a 2- to 4-fold decrease in transferable 2,4-D residues across turfgrasses and with modified California roller and hand wiping methods, while transfer decreased 36-fold from soccer ball roll. This decline agrees with Jeffries *et al.*[14], who reported 2,4-D transfers via soccer ball roll decreased from 11.2% of the applied immediately after application on hybrid bermudagrass to 0.3% after a 1 h drying period. These data emphasize the effect sample collection time and method applied have in quantifying transferable residues from turfgrass. A soccer ball roll is a relatively specific process in turfgrass systems, and while it provides pertinent information for human exposure on an athletic field, it may not be as appropriate to solely utilize for general human exposure risk assessments as other methods.

[Place Table 1 here]
[Place Figure 5 here]

Data from 1 and 3 DAT suggests sample collection methods do not transfer 2,4-D from treated vegetation similarly across turfgrass species, which was hypothesized due to varying canopy dynamics. Within a sample collection method at 1 DAT, 2,4-D transfers from hybrid bermudagrass (17.3 to 31.2% of applied) was greater than creeping bentgrass (10.6 to 16.2%) and tall fescue (8.1 to 20.9%), which is likely due in part to varying canopy dynamics and herbicide-physiological effects across species (Table 2). Elucidating this occurrence is beyond the scope of this experiment; however, it is highlighted to demonstrate the importance of turfgrass species selection for transferable pesticide residue research. Transferable 2,4-D did not vary between methods on creeping bentgrass at 1 or 3 DAT, which was the finest textured, lowest mown turfgrass evaluated. This allowed for relatively consistent sorbent material-treated vegetation contact across the three evaluated methods. 2,4-D transfers varied across methods in hybrid bermudagrass and tall fescue, with hand wiping resulting in the greatest transfer. Hybrid bermudagrass and tall fescue are coarser textured than creeping bentgrass, and were mown at higher heights (5 and 9 cm, respectively), which accentuates an inherent limitation of rolling-based methods of laying vegetation over (Figure 6). When this occurs, sorbent material-treated vegetation contact can be reduced and consequently, underestimate transferable residues.

[Place Table 2 here]
[Place Figure 6 here]

Table 1. Transferable 2,4-D from field plots the day of application.
Main effect of turfgrass species and sampling method on transferable 2,4-D data reported as percent of the initial application rate. Sample collections occurred immediately following application and after a 1 h drying time.

Table 2. Transferable 2,4-D from field plots 1 and 3 days after application.
Turfgrass species-by-sampling method interaction on transferable 2,4-D data reported as percent of the initial application rate. Sample collections occurred at 7:00:00 eastern standard time.

Figure 1. Hand wiping pressure quantification.
Approach used to quantify pressure from fronthand (A) and backhand (B) wiping motions. To do so, spray a green food coloring solution (water + 1% v/v nonionic surfactant) over a nonporous surface (ex. glass or metal tray) and press hand as intended for pesticide dislodge sampling. Quantify the contact surface area by digital image analysis as described by Campillo *et al.*[17] to determine proportion of green pixels per image of known area. Collect a turfgrass core from the intended research area when soil is at field capacity and secure it to a digital weight scale. Quantify the mass of downward force when hand wiping. Hand wiping pressure should not exceed 2 kPa.

Figure 2. Hand wiping directions.
Progression is intended to contact maximum unique treated vegetation surface area to glove. Two gloves are required to avoid overloading cotton when sampling $\leq$ 415 cm^2 area; however, this should be confirmed in pesticide- and site-specific conditions prior to experiment initiation.

Figure 3. Soccer ball roller.
Excluding A to B, all junctions are glued with PVC adhesive. Part C length can vary by supplier due to varying dimensions of parts B and D.

Figure 4. Turfgrass core collection.
Golf course cup cutter is a robust, relatively cheap apparatus for turfgrass/soil collection. Remove the inside plunger used to eject cores prior to use for transferable pesticide residue sample collections so that vegetation is not inadvertently contacted, which may reduce pesticide residue concentrations.

Figure 5. Turfgrass systems evaluated.

Turfgrass canopy density and height can vary by system. Within the presented research, density (highest to lowest) ranked creeping bentgrass (A) > hybrid bermudagrass (B) > tall fescue (C); while height (highest to lowest) ranked tall fescue > hybrid bermudagrass > creeping bentgrass.

Figure 6. Tall fescue canopy following modified California roller sampling.
An inherent limitation to this transferable pesticide residue sampling method is increasing potential for grass blades to lie down as canopy height increases. When this occurs, abaxial surfaces are not contacted by the cotton sorbent sheet, thus, potentially underestimating transferable residues.

DISCUSSION:
Regulating agencies have not identified a specific method to quantify transferable pesticide residues from turfgrass. This research supports utilizing different methods based on site- and exposure process-specific criteria, as they all have utility for human risk assessments. However, they all have limitations that researchers should be cognizant of prior to their use. Lastly, turfgrass species is not a site selection parameter currently stated in transferable pesticide residue protocols, and this research builds on previous efforts suggesting its inclusion should be stated.

The described soccer ball roll method is relatively robust across samplers and only requires one individual to complete. It also mimics a very common occurrence in the most popular international sport, and has many implications for nondietary pesticide exposure from hand-to-mouth contact. Additionally, this concept could be applied with minimal modification for pesticide transfer onto objects associated with other sports. However, it is a comparably less aggressive method for pesticide transfer and consequently, should not be solely utilized in risk assessments.

Of the three methods presented, the modified California roller has been employed most extensively in turfgrass transferable residue research. It is the most robust approach across samplers, and transferred a similar amount of 2,4-D as other methods at the lowest mown turfgrass system evaluated, creeping bentgrass. This suggests that this method should be employed for transferable pesticide residue research on golf course putting greens, and potentially other closely mown systems such as golf course fairways/tees and athletic fields. The limitations of this method include potentially laying grass blades down as mowing height increases and the requirement of three individuals to complete. Additionally, the roller and frame preparation required between samples can be time consuming, and previous research has shown pesticide transfer fluctuates over relatively short timescales within a day as canopy moisture dissipates[14]. This may limit the amount of samples that can be collected at a given timing (i.e. reduced treatments), or add a confounding factor to data should samples be collected over an extensive amount of time. Researchers should be aware of this as they plan experiments utilizing the modified California roller.

Hand wiping over treated plant vegetation with cotton-based gloves is a method commonly used to measure transferable pesticide residues for workers in orchards and tobacco due to the high frequency of hand-to-vegetation contact associated with agricultural production. While this method has been utilized less in turfgrass systems, it provides a superior approach to quantify transferable residues in turfgrass systems at mowing heights commonly associated with commercial/residential lawns and parks. Additionally, hand-to-treated turfgrass contact is common for both nonoccupational and occupational risk assessments, as turfgrass systems are utilized for a variety of societal purposes. Of the three evaluated methods, hand wiping is the least reproducible method across samplers, which may require additional measures (replications, training, etc.) to produce conclusive results.

Although the evaluated methods vary widely in their execution, the critical steps within each protocol conceptually overlap. Sampling at a constant speed and pressure is paramount to produce reproducible data, as these influence sorbent material-pesticide transfer. Maintaining a constant speed is required of samplers across all three methods, while pressure is a point of concern for hand wiping only. Samplers should not put additional pressure on the modified California or soccer ball rollers, while hand wiping is an approach that takes substantial preliminary efforts to maintain consistency within, and across samplers. This is the greatest limitation to transferable residue research relying solely on hand wiping, and future research should identify a less subjective approach that provides its unique attribute of canopy penetration while minimally laying grass down.

The purpose of collecting turfgrass vegetation is to provide a reference point in addition to the amount of pesticide initially applied by accounting for dissipation between application and subsequent sample collection timings. Furthermore, quantifying pesticide residue in vegetation enhances explanation when nondetection occurs in transfer samples. Basically, it allows the researcher to determine if transfer did not occur because pesticide residue was sorbed in/on vegetation, making it nontransferable, or if residue was no longer detectable in/on vegetation.

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The authors have no disclosures to make.

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Figure

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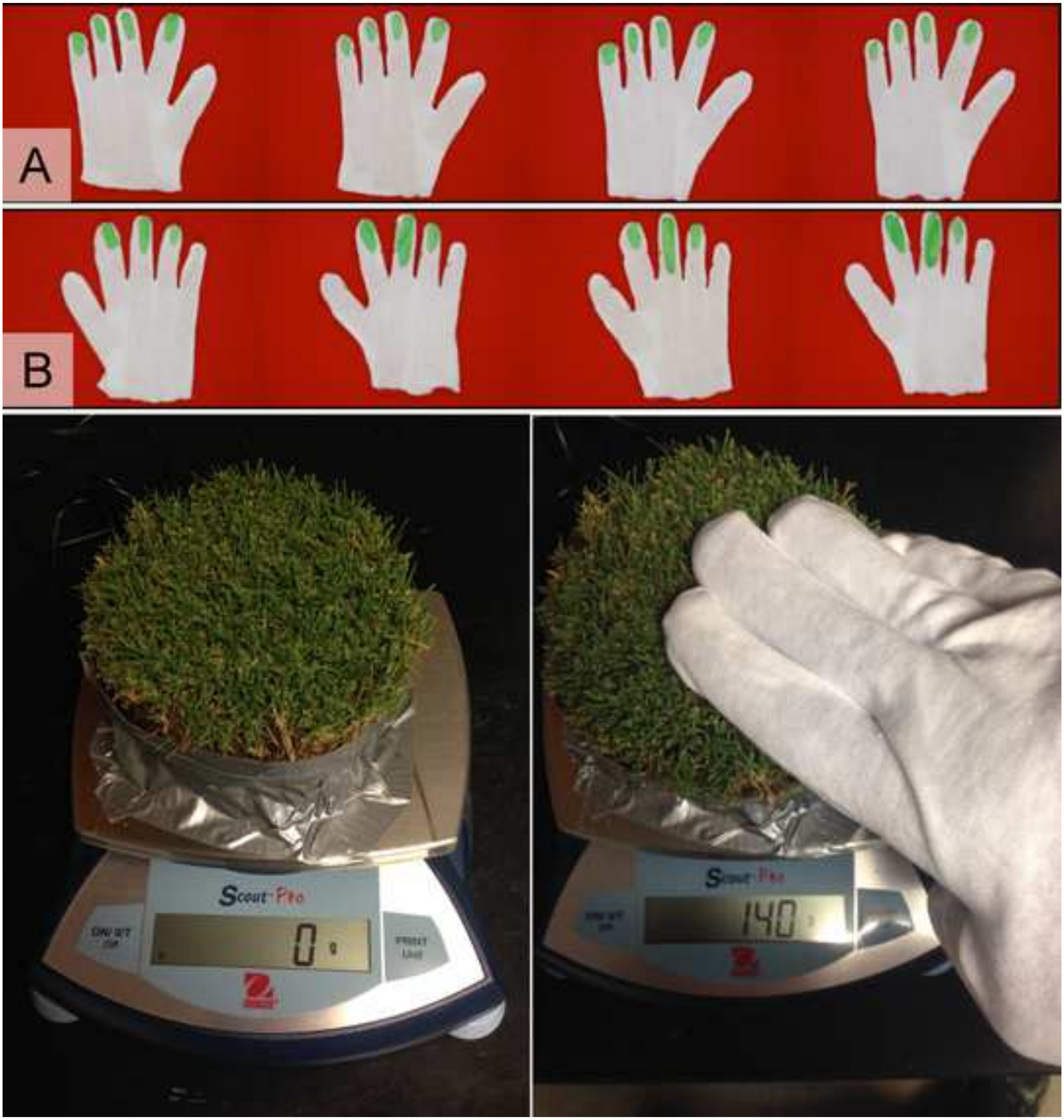


N
W
E
S
Glove 1
1.) Fronthand – North to South
2.) Backhand – East to West
3.) Fronthand – Perimeter Clockwise
Glove 2
1.) Fronthand – East to West
2.) Backhand – North to South
3.) Fronthand – Perimeter

Figure

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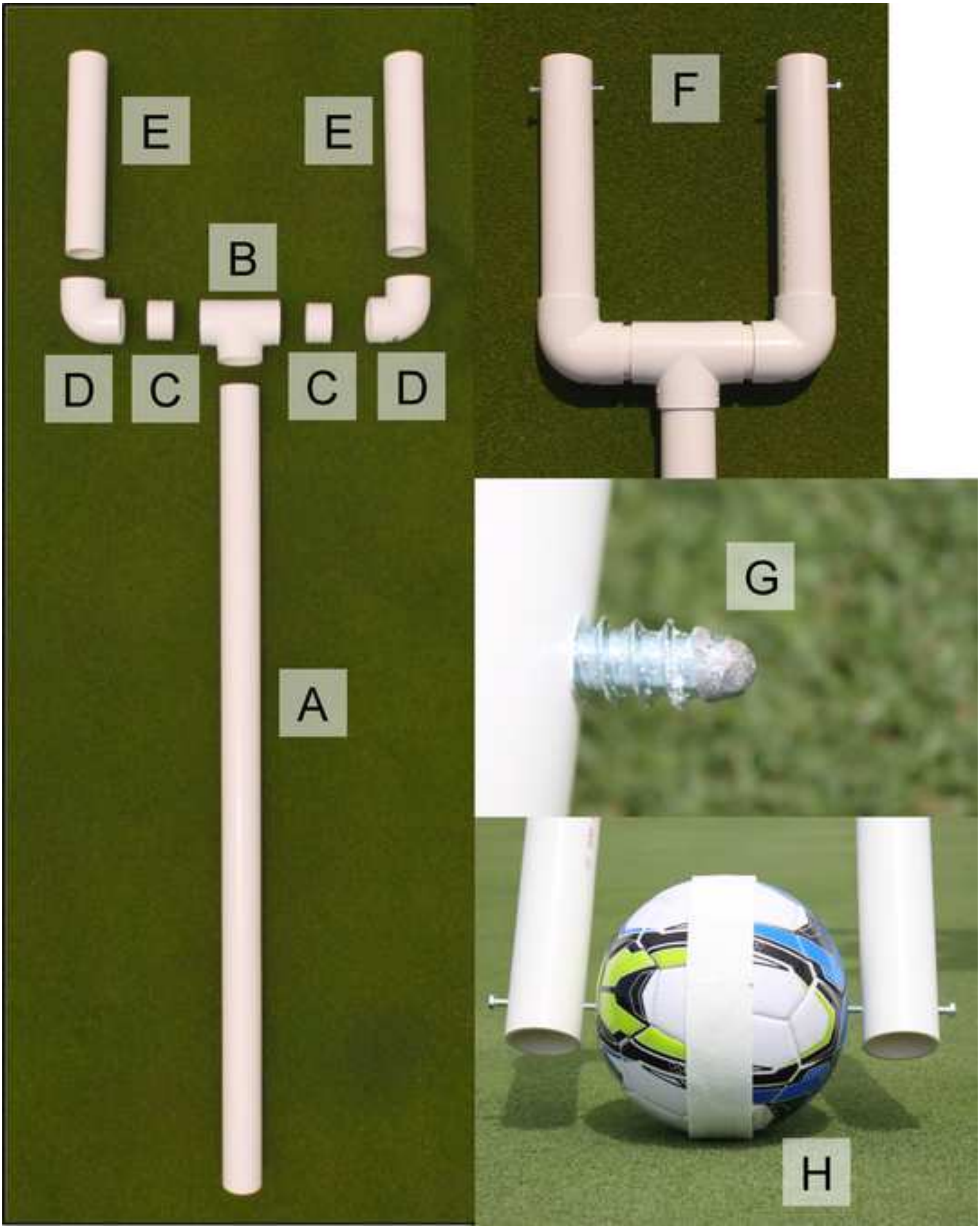

Figure

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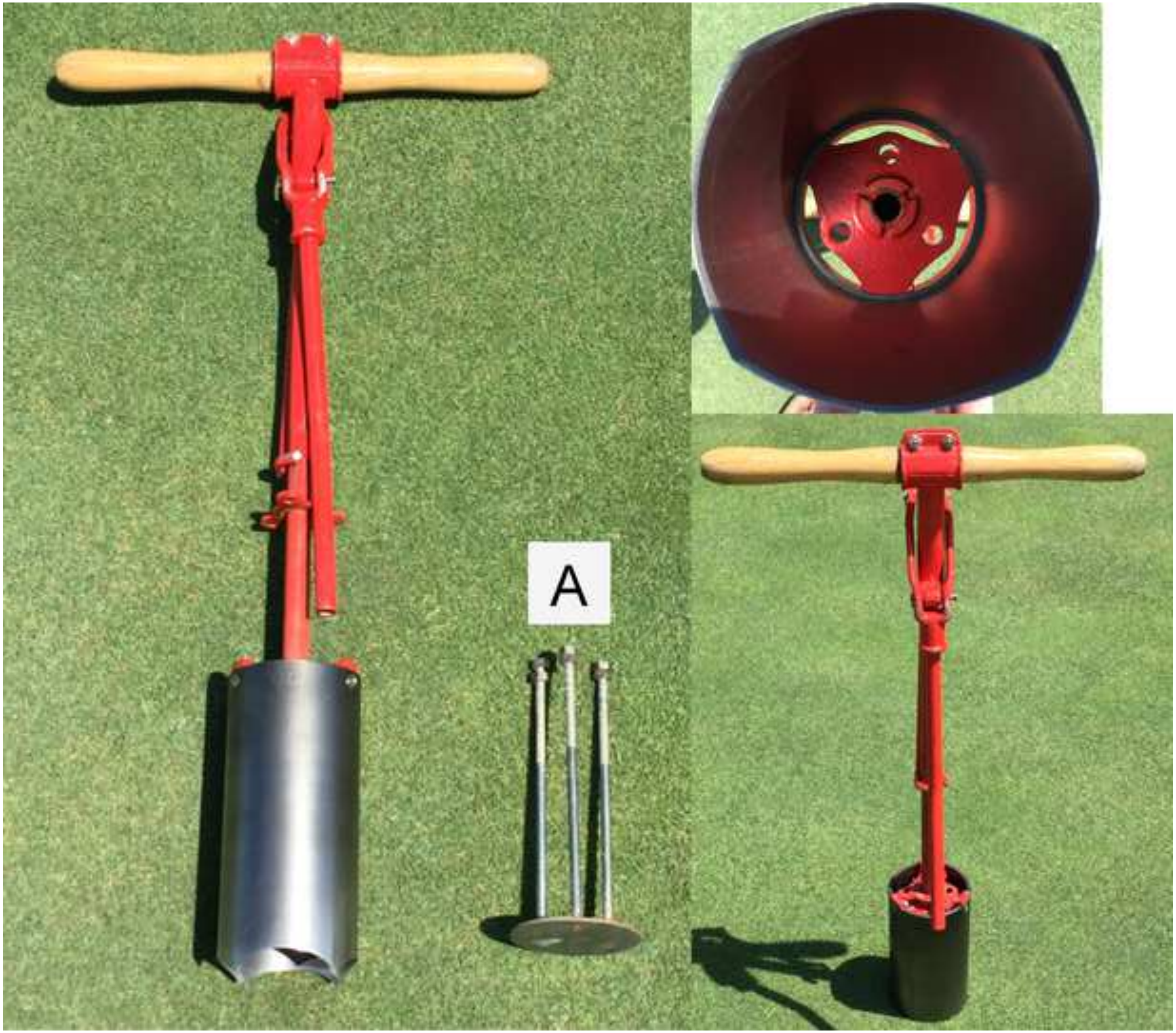

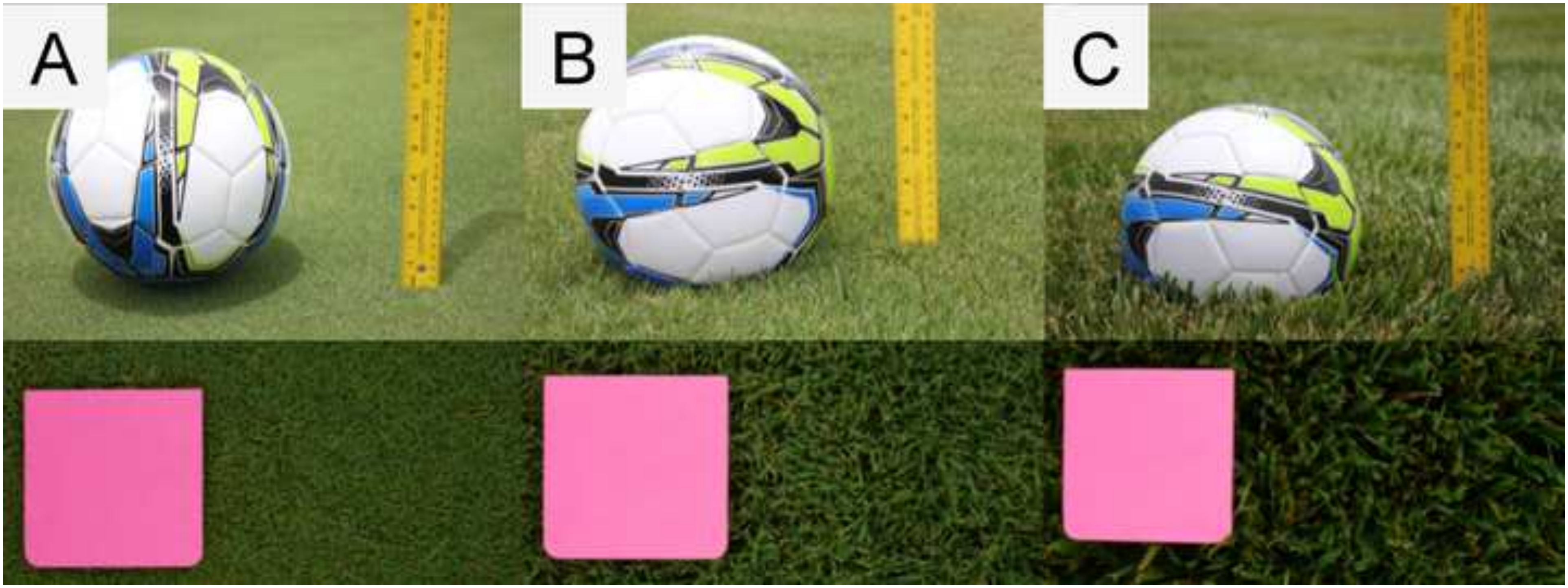
A
B
C

Figure

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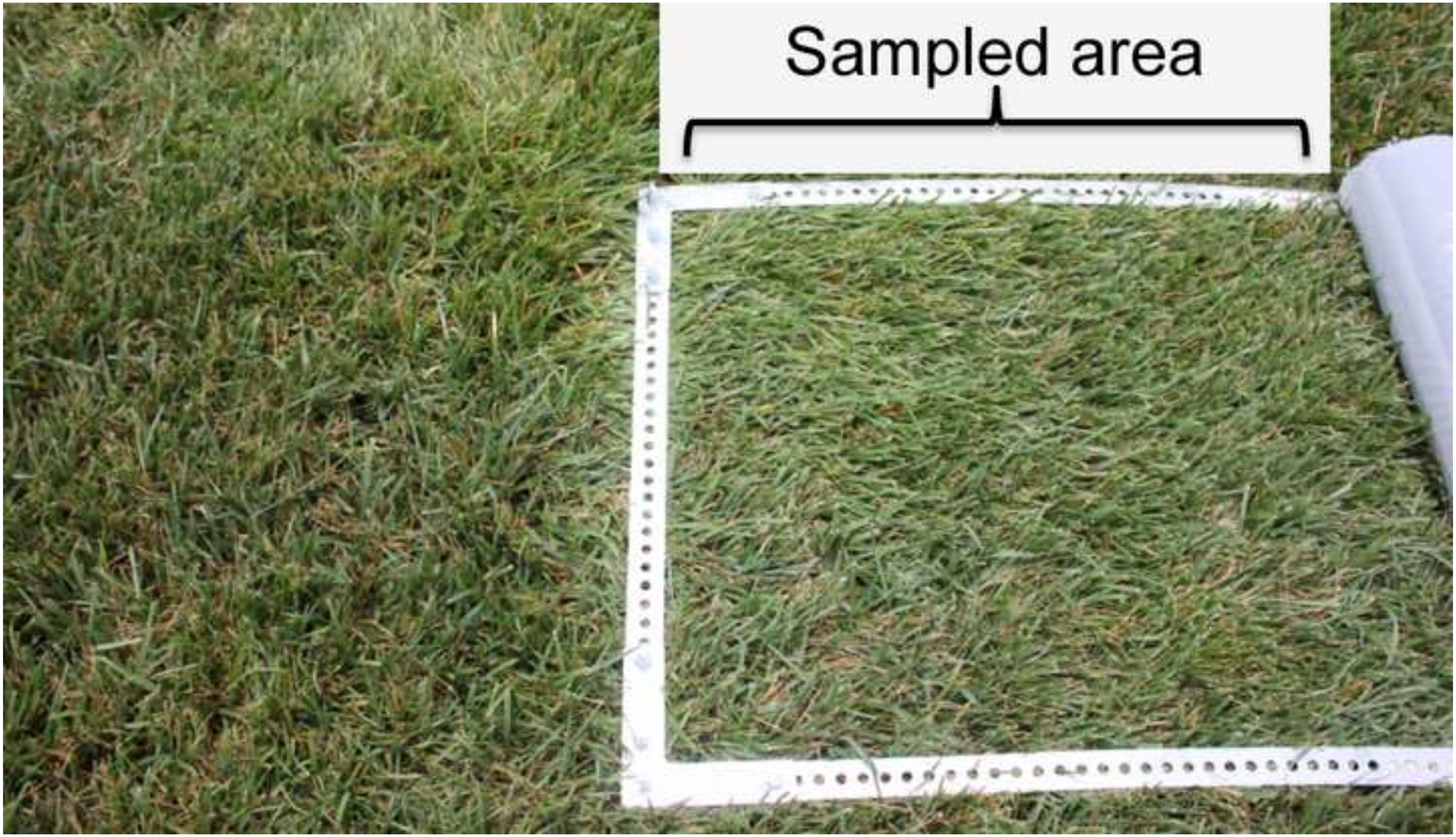

Table 1

	Creeping bentgrass	Hybrid bermudagrass	Tall fescue	Ball roll	Mod. Cal. roller	Hand wipe
	% dislodged of applied 2,4-D					
0 DAT - 0 hr	21	16.4	15.1	14.4	16.8	21.2
$LSD_{0.05}$		2.8			2.8	
0 DAT - 1 hr	5	6.8	4.9	0.4	7.7	8.5
$LSD_{0.05}$		1.0			1.0	

Turfgrass	1 DAT - 7:00 EST Ball roll	Mod. Cal. roller	Hand wipe	$LSD_{0.05}$
	% dislodged of applied 2,4-D			
Creeping bentgrass	10.6	13.6	16.2	NS
Hybrid bermudagrass	17.3	20.9	31.2	2.2
Tall fescue	8.1	9.1	20.9	2.7
$LSD_{0.05}$	3.2	3.2	5.2	

Turfgrass	3 DAT - 7:00 EST Ball roll	Mod. Cal. roller	Hand wipe	$LSD_{0.05}$
	% dislodged of applied 2,4-D			
Creeping bentgrass	1.9	2.8	3.1	NS
Hybrid bermudagrass	1.9	4.8	7.6	2.1
Tall fescue	1.8	2	6.8	2.6
$LSD_{0.05}$	NS	0.9	3.4	

Item	Company	Catalog Number
General	---	---
Nitrile gloves	Any	NA
Coolers	Any	NA
Turf paint	Any	NA
Field plot measuring equipment	Any	NA
Protective foot apparel	Any	NA
Whatman 3 MM Chr Chromatography Paper	Fisher Scientific	05-716-3E
Ball roll	---	---
PVC pipe (5 cm inner diameter)	Home Depot	531137
Hacksaw for PVC cutting	Any	NA
90 degree elbow (5 cm inner diameter)	Home Depot	RCE-2000-S
Tee coupler (5 cm inner diameter)	Home Depot	PVC024001600HD
PVC adhesive	Any	NA
Lag bolt (0.6 cm diameter by 7 cm length)	Home Depot	801366
Size 4 soccer ball	Any	NA
Pressure gauge	Any	NA
Hand air pump	Any	NA
Fabric scissors	Any	NA
Scott Rags-In-A-Box	Uline	S-12809
Adhesive tape	Any	NA
8 oz sealable glass jar	Any	NA
Hand wipe	---	---
100% cotton heavyweight inspection gloves	Uline	S-19284
Digital camera	Any	NA
ImageJ software	National Institutes of Health	https://imagej.nih.gov/ij/
Digital scale that measures up to 400 g	Any	NA

Item	Company	Catalog Number
Stopwatch	Any	NA
Plastic bucket (23 cm diameter)	Home Depot	209313
16 oz sealable glass jar	Any	NA
Modiifed California roller	**---**	**---**
Metal conduit (1.25 cm diameter by 1.8 m length)	Home Depot	101543
PVC pipe (10 cm inner diameter)	Home Depot	531103
Sand + reebar to bring roller to 14.5 kg	Any	NA
PVC plug	Home Depot	33403D
Polyurethane foam sheet (1.25 cm thick) to cover PVC pipe	Any	NA
6 mm painter's plastic	Any	NA
Plexiglass sheet (107 cm length by 76 cm width by 0.6 cm thick)	Any	NA
Toggle clamps	Any	NA
Metal nails (10 to 15 cm length) to secure frame to ground	Any	NA
100% cotton sheets (> 200 threadcount)	Any	NA
Tweezers	Any	NA
32 oz sealable glass jar	Any	NA
Turfgrass vegetation core collection	**---**	**---**
Lever action golf course cup cutter	Par Aide Product Company	1001-1
Knife	Any	NA
Fiskars Herb Scissors	Home Depot	96086966J
Turf plug plastic container	Any	NA

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Author(s): Matthew D. Jeffries, Travis W. Gannon and Patrick J. Maxwell

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The manuscript has been modified by the Science Editor to comply with the JoVE formatting standard. Please maintain the current formatting throughout the manuscript. The updated manuscript (55182_R1_071216.docx) is located in your Editorial Manager account. In the revised PDF submission, there is a hyperlink for downloading the .docx file. Please download the .docx file and use this updated version for any future revisions.

1. Formatting: 5.1, 5.2, 5.3 – Method only should appear in the heading. The number of people required can be listed as a note.
Revised as a note

2. Grammar:
-Line 34 – "suitability…are discussed"
Revised

-2.3.1, 4.2.1 – Please use imperative tense or convert to a note.
Revised

-5.2.5 – "takes"
Revised

-5.2.6 – "removes"
Revised

-6.2.3 – "should shallow enough space'
Revised

3. Additional detail is required: 5.1.5, 5.3.6.1 – When should samples be transferred to the freezer?
Revised to say within 3 h of sample collection

4. Results: Please convert data from one of the tables to a graph to provide visual interest for the video.
1 day after treatment data from Table 2 converted to a graph. Submitted as a PDF tiled "1 DAT data" under "Supplemental File"

Reviewers' comments:
Reviewer #1:
Manuscript Summary:
The authors do a nice job of explaining the purpose of this research as well as relating how the different methods can alter findings regarding pesticide transfer.
Thank you

Major Concerns:
There are no major concerns with the article. The edits suggested are to improve flow or specificity with a sentence.

Minor Concerns:
Short Abstract
No edits

Long Abstract
P1L38 remove inherently replace with can
Revised

P1L43 Experimental approaches, often used to assess pesticide transfer to humans, including…
Manuscript revised to "Experimental approaches used to assess pesticide transfer to humans including hand wiping with cotton gloves, modified California roller (moving a roller of known mass over cotton cloth) and soccer ball roll (ball wrapped with sorbent strip) over three treated

turfgrass species (creeping bentgrass, hybrid bermudagrass and tall fescue maintained at 0.4, 5 and 9 cm, respectively) are presented."

P1L48 change aggressive to describing how it differs as an approach
The authors believe this is already included in this section with "The modified California roller is the most extensively utilized approach to date, and is best suited for use at low mowing heights due to its reproducibility and large sampling area. The soccer ball roll is a less aggressive transfer approach; however, it mimics a very common occurrence in the most popular international sport, and has many implications for nondietary pesticide exposure from hand-to-mouth contact. Additionally, this approach may be adjusted for other athletic activities with limited modification. Hand wiping is the best approach to transfer pesticides at higher mowing heights, as roller-based approaches can lay blades over; however, it is more subjective due to more variable sampling pressure."

Introduction
L63-65 an awkward sentence - consider breaking into two sentences. Remove the word stand.
Manuscript revised to "They provide many positive societal attributes including dust control, heat dissipation, recreational surfaces and soil stabilization. However, pest encroachment may occur which requires the use of pesticide(s) to maintain the turf to an acceptable level[3]."

L67 remove inherently
Revised

L70 remove which can then transfer to humans.
Revised

L72 U. S.
Revised

L73 replace contaminated with treated
The authors request leaving "contaminated", as this was the term used in the reference.

L75 remove remaining
Revised

L85 remove (across and within users)
Revised

L89 remove additionally
Revised

L92-93 remove the parenthesized information. Differences of plant parameters can be discussed later in detail.
Revised

L101 remove ultimately. …systems may limit the implementation…
Manuscript revised to "Ultimately, variability between turfgrass systems inhibits the implementation of a universal method to quantify transferable foliar pesticide residues. Therefore, method selection to optimize human risk assessments should encompass pesticide-, process-, site- and species-specific criteria."

This revision also applies to the following L 101-104 revision.

L101-104 split the sentence. Good information but a runon
Revised

L104 change is to was
Revised

Protocol
1.2) could you not use records for this; or at least state that if the area of interest does not have the appropriate records to indicate pesticide applications then sampling is required.
Researchers may be able to circumvent sampling if records are adequately kept, and the field persistence of the compound is exceptionally well understood. However, pesticide field persistence data often varies by an order of magnitude across site-specific conditions, and researchers are going to have to take a fairly high-risk guess that the compound they are researching is no longer detectable (assuming it had been applied over the area historically). Ultimately, adding these preliminary samples is advisable in most scenarios, and does not add an appreciable workload to the project (typically six total preliminary samples; and these projects typically produce 200 to 600 samples depending on the treatment structure). The authors request not including the comment regarding pesticide application records.

2.1) remove the parenthesis. … prior to the initiation of the experiment mow turfgrass species to the appropriate height and collect clippings.
Revised

2.2.1) remove …creating the worst-case scenario for subsequent transfer
Revised

Note: include that the proper protective equipment should be worn by applicators and nonapplicators near the test area.
Revised

2.3.1) would precipitation possibly wash the active ingredient off the leaf surface?
More than likely, yes.
Manuscript revised to "Note: Plots are covered to prevent pesticide wash from treated vegetation into turfgrass thatch and/or the soil surface, making it less dislodgeable and underestimating exposure risk."

2.3.1.1) why not conduct this over a period in which there is no precipitation expected. Also some chemicals breakdown through photolysis - Covers would limit this process and possibly overestimate transfer.
This would be ideal, but just not logistically possible in the region research is conducted. Predictable six day periods in spring to summer are rare, which is compounded when trying to repeat research over time.

3.3) use the word control for checks. …applied to the turf
Revised

3.4) as described by… remove inherent …as to identify potential sources of contamination
Revised

4.1) ….and again once the pesticide has dried on the leaf surfaces. Depending on environmental conditions this is typically between 1 and 3 hours post pesticide application.
Revised

Note: …replace as often as needed to prevent contamination
Revised

5.1.1.2 Primarily use fingers to contact turfgrass leaves while limiting leaves from laying over.
Revised

5.1.1.2.1 remove parenthesis and incorporate into sentence
Manuscript revised to "5.1.1.2.1) Prepare a 10% v/v water-based solution of green food coloring and a 1% v/v nonionic surfactant, and spray on a nonporous surface to create a thin film.

Note: The nonionic surfactant is used to increase solution coverage over the nonporous surface by reducing surface tension."

Representative Results
L362 awkward sentence May just want to state pesticide residues methods within turfgrass systems. This would allow the stance that the systems change due to species, soil, maintenance, etc…
The authors acknowledge there is a lot going on this sentence, but the intention of it was to highlight that comparing transfer methods across turfgrass systems has not been documented before. Highlighting that these methods respond differently across systems is the second objective of the manuscript (first objective being how to conduct this research). The authors have included the term "single" in the sentence to improve clarity.

Manuscript revised to "Building on previous research efforts comparing transferable pesticide residue methods within a single turfgrass system, and turfgrass systems within a single transferable pesticide residue method, a field study (initiated May 24, 2016 in Raleigh, North Carolina, USA) was conducted to compare methods across turfgrass systems."

Also consider breaking the sentence into two sentences for easier reability
L371-372 remove parenthesis and incorporate information into sentence
Revised

L376 covering? focused on or examining or evaluating
Revised to "evaluating"

L383 …compared to other transfer techniques such as…
Revised

L386 remove drastic
Revised

L388 These data emphasize the effect sample collection time and method applied have in quantifying….
Revised

L391 …field, it may not be as appropriate to fully characterize general human…
Revised

L397-402 How do the canopy dynamics affect the results. Please tie it together for the reader. Especially for nonturf grass readers. I believe a discussion here is very relevant to your case why different results and interpretations vary across the method employed.
Manuscript revised to "Transferable 2,4-D did not vary between methods on creeping bentgrass at 1 or 3 DAT, which was the finest textured, lowest mown turfgrass evaluated. This allowed for relatively consistent sorbent material-treated vegetation contact across the three evaluated methods. 2,4-D transfer varied across methods in hybrid bermudagrass and tall fescue, with hand wiping resulting in greatest transfer. Hybrid bermudagrass and tall fescue are coarser textured than creeping bentgrass, and were mown at higher heights (5 and 9 cm, respectively), which accentuates an inherent limitation of rolling-based methods of laying vegetation over (Figure 6). When this occurs, sorbent material-treated vegetation contact can be reduced and consequently, underestimate transferable residues."

L406- great information and explaination
Thank you

L460 remove to date split sentence into two sentences.
Manuscript revised to "Regulating agencies have not identified a specific method to quantify transferable pesticide residues from turfgrass. This research supports utilizing different methods based on site- and exposure process-specific criteria, as they all have utility for human risk assessments."

L462 edit sentence to allow for the positives as well as limitations of each method. I agree researchers must be aware of these benefits and limitations.
The purpose of this paragraph is to summarize some of the main points of the research not specific to how to conduct such research. The authors believe the positives and limitations of each method is adequately covered in the following three paragraphs, where each method is discussed in more detail. Discussing at this point as suggested would be redundant if stated twice, or more confusing for the reader if all methods are discussed simultaneously. Therefore, the authors request leaving the discussion as originally submitted.

Do not use the word aggressive.
Merriam-Webster defines aggressive as "more severe, intensive, or comprehensive than usual especially in dosage or extent", which we believe adequately describes the presented transfer methods interaction with the turfgrass canopy. The authors believe this will be more evident when video is provided for the reader to visualize these methods, and request using the term "aggressive" in the manuscript as originally submitted.

Explaining each method is a good way to characterize their positives and limitations.
Thank you

L485 reduced
Revised

L503 remove robust data replace with reproducible
Revised

L509 remove parenthesis and incorporate into sentence.
Would it be a good idea for technology to be developed to allow for constant pressure and repeatability?
Yes, it would be ideal for technology to address this, and is something our program is actively investigating.

Manuscript revised to "This is the greatest limitation to transferable residue research relying solely on hand wiping, and future research should identify a less subjective approach that provides its unique attribute of canopy penetration while minimally laying grass down."

L517 remove parenthesis information
Revised

References
Appropriate

Photos
I really like the photos. Well done.
Thank you

Graphs
Appropriate and simple

Additional Comments to Authors:
N/A

Reviewer #2:
Manuscript Summary:
This is a very interesting paper talking about a detailed protocol for quantifying the foliar pesticide residues and the valid results based on the experiment. The main method on how to quantify the pesticide residues is addressed very detailedly. The experiment was set up using fairly practical

materials and design so it can be easily repeated by other researchers, which is the key value of this research. The diagram of the instrument design is clearly demonstrated and explained.
The authors mentioned different methods used in previous studies briefly. However, a more detailed comparison is preferred to let other researcher decide which method is best for different practice. So more information on alternative approaches should be address in the Introduction section. This is a main drawback of this manuscript.
The authors agree with Reviewer 2 that information regarding transfer methods outside of the three specifically covered in this manuscript is limited (other than references 9-12 which provide more detail). However, covering methods outside of the hand wipe, modified California roller, and soccer ball roll is somewhat outside of the realm of this manuscript, especially considering the 6 paragraphs Introduction limit per journal instruction. We feel that the information provided throughout this manuscript accomplishes our objectives to provide information for researchers to utilize the three transfer processes covered in detail, and also highlight that researchers need to consider site conditions and the transfer process of interest when selecting a method(s). For example, the soccer ball roll described is adequate for this process and relatively light impact transfer processes, but has limited utility for an adult soccer player sliding, which would probably be better suited for a drag slide approach that is not even covered in detail in the manuscript. By providing results evaluating three methods, across three turfgrass species, and discussing factors that resulted in differing 2,4-D transferability, we feel that this latter objective was accomplished. Ultimately, the authors request omitting additional information on other transfer methods.

Major Concerns:
N/A

Minor Concerns:
Line 122, the authors mentioned that the minimum dimension of the plots. The authors didn't address where does this number come from. Please insert some explanations or citations.
Manuscript revised to "Note: Plot dimensions can vary based on site-specific conditions, but should be a minimum of 3.3 m^2. This will allow for a 3.7 by 0.5 m ball travel pathway, as well as an equivalent adjacent area for foot-traffic and vegetation sampling."

Additional Comments to Authors:
N/A

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Supplemental File (as requested by JoVE)
1 DAT data.pdf