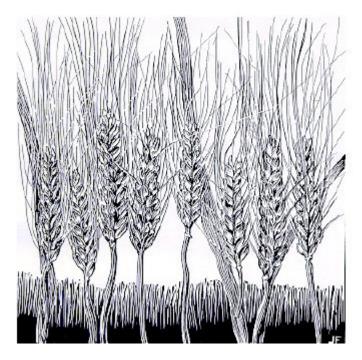
# NATIONAL KARNAL BUNT SURVEY PROCEDURES



[Drawing of wheat heads by Joel Floyd, retired USDA employee]

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# **Background**

Karnal bunt (KB) is caused by the fungus *Tilletia indica* Mitra. This plant pathogen was first reported in 1931, infecting wheat growing near the city of Karnal in the Indian state of Haryana – hence the common name "Karnal bunt". The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection Quarantine (PPQ) National Karnal Bunt Survey was initiated in 1996 in response to the detection of KB disease in the United States (U.S.). Since then, this survey has provided invaluable information in the form of negative occurrence data to support the export of U.S. wheat to foreign markets. USDA-APHIS-PPQ continues to explore new survey, detection, control, and analysis methods for the KB program. As new information becomes available, PPQ will update these survey protocols and associated program guidance and regulations.

# **Purpose**

The purpose of the National KB Survey is: (1) to provide the basis for U.S. certifying officials to issue phytosanitary certificates and additional declarations, if required, by the countries to which we export (or may export) wheat, and (2) to establish a record of KB pest free areas in the United States. The survey also functions as a valuable surveillance tool, enabling rapid response by APHIS-PPQ and its partners in the event of a new detection.

### Why the KB program and participation in the KB National Survey are important

The cooperative KB program is a vital part of the U.S. agriculture safeguarding continuum and is consistent with the mission, vision, and strategic goals of PPQ. The KB program and national survey protect the health and value of U.S. wheat, durum wheat, and triticale. In a highly competitive global marketplace, the United States is one of the world's leading wheat exporters: more than 40% of the countries that imported U.S. wheat in 2021 consider KB a quarantine pest and maintain trade restrictions for it. The National KB Survey helps protect these U.S. exports by providing the survey data to certify that shipments have originated in U.S. KB-free areas. It also advances the well-being of U.S. consumers by ensuring the continued availability of affordable, high-quality wheat products in the domestic marketplace.

Overall, the KB program and national survey help safeguard 1.8 billion bushels and 44 million acres of wheat production across the United States, valued at \$9.3 billion (USDA NASS, 2020). In 2020, the U.S. exported 26.055 million metric tons of wheat valued at \$6.3 billion; 128,000 metric tons of wheat products valued at \$171 million; and 277,000 metric tons of wheat flour valued at \$153 million (FAS GATS, 2020).

# **National Survey Areas**

The National KB Survey covers wheat-producing states in areas where KB is currently not known to occur.

# Crops to be Surveyed

Wheat (*Triticum aestivum* L.), durum wheat (*Triticum durum*), and triticale (*Triticum aestivum* L. x *Secale cereale* L.) from producing counties.

# **Protocols for Collecting Grain Samples**

#### **General Intent and Design**

The National KB Survey collects representative samples from counties where susceptible host crops are produced according to statistics provided by USDA, National Agricultural Statistics Service (NASS). The number of samples is based on the average production of each host for the most recent 5-year period, which may be obtained through the NASS website: www.nass.usda.gov/. Refer to Appendix A for detailed instructions.

The survey does not intend to sample every local elevator or farm, but to represent crop production within overall significant production areas. Significant production means an area that produces over 1 million bushels in a 5-year average. An area may be defined as a single county or as several counties that, when combined in a composite sample, meet the million-bushel production mark. One survey sample should be submitted for each million bushels of production. However, no county should be surveyed more frequently than every other year.

#### Sample Size and Retention

The official sample size for the bunted kernel analysis is **four (4) pounds** (1.814 kgs). APHIS-PPQ requests that all samples submitted to the National KB Sample Processing Laboratory <u>meet but not substantially exceed</u> that weight. **Any samples received that weigh less than 4 pounds will not qualify as official for the purpose of this survey**.

A Sample Information Form (Appendix B) should be filled out for each individual sample at the time of collection. The person submitting the sample should also retain an additional 250-gram sub-sample (8.818 ounces), stored in a Nalgene® bottle or similar appropriate container. This sub-sample should be cross-referenced to the source sample, in case additional testing becomes necessary. If the sample is a composite of grain from multiple counties, a 250-gram reserve sample from <a href="mailto:each">each</a> of the counties should be held separately when possible. That way, as applicable, further testing of those sub-samples can narrow down a KB-positive sample to the individual county that actually produced the bunted kernel.

Once the official 4-pound sample has been found free of KB, the 250-gram reference sample may be disposed of in the regular trash. If a bunted kernel <u>is</u> found in the 4-pound sample, any unused portion of the 250-gram sub-sample must be disposed of using an APHIS-approved method, as follows:

#### 1. Autoclave

- Temperature: 250 degrees Fahrenheit (121 degrees Centigrade)
- Pressure: 15 pounds per square inch
- Time: minimum of 30 minutes
- Heat: steam heat
- To ensure adequate heat penetration, restrict grain depth to 2 inches when treating loose in trays. When treating in packages, restrict individual package weight to 5 pounds (or less) and leave ample spacing in between packages.

#### 2. Incineration

 Incineration to ash at an incinerator that is signed to a valid PPQ compliance agreement.

#### 3. Landfill

• Deep burial at a landfill that is signed to a valid PPQ compliance agreement.

# **Timing of Survey Sampling**

The ideal time to sample grain for KB is soon after harvest; however, grain samples may be collected at any time. An important point to remember is that the longer after harvest the samples are collected and analyzed, the greater the chance of having to regulate a bigger area if a sample tests positive, owing to downstream grain movement that may have occurred in the meantime. Trace-back and trace-forward efforts also tend to become more difficult as time passes. Samples can be taken from local elevators before the harvest is complete if the samples are representative of the county in which they were taken and otherwise meet the minimum requirements in this sampling protocol.

### Sampling Intensity

Samples should be collected in parallel with the wheat harvest and in proportion to host production from producing counties. Counties with KB-free status should be sampled only every other year. A yearly survey can be maintained by sampling one half of the state's wheat-producing counties each year. As noted above, the sampling requirement is one 4-pound sample for each 1 million bushels of production in counties meeting that production threshold. Multiple counties may be combined to reach the million-bushel production mark, in cases where the counties do not reach that level on their own. When possible, single-county samples will preferably include grain from at least 10 different individual producers to ensure an adequate scope of representation for the county.

#### **Sampling Methods**

Samples taken at points of aggregation near their production areas (local elevators) are a practical source of host material for the survey and usually represent the best crop profile. Aggregation-point samples will generally be a composite of grain from many producers across a given county, and in some cases across multiple counties. Each individual sample, however, should consist entirely of one species: grain of different KB-susceptible

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hosts should not be mixed.

If a county has more than one elevator where grain is aggregated, then the elevator chosen for sampling should be the one that best represents the entire county. If a county appears in the NASS data but has no elevator, samples for that county should be taken from the elevator(s) to which the grain is shipped. This may require sampling at elevators in adjacent counties, or even in adjoining states.

At the beginning of the year, the State KB Survey Coordinator should create a list of all elevators to be surveyed in the state, with the contact person identified at each location. In states where PPQ is not the survey leader, a PPQ representative should visit participating elevators on an occasional basis to ensure overall integrity of the sampling process, provide information, and answer questions concerning the program.

Each elevator normally takes a moisture and/or quality sample from each arriving load. If arrangements can be made with the elevator to save all (or most) of these samples by placing them in a barrel, national survey samples may be taken when each barrel reaches capacity. This approach will represent a sampling of all (or most) of the wheat coming into the elevator. The objective is to take a 4-pound sample (adding 250 grams more for the reference sub-sample) by using either a small grain probe or a plastic cup to draw grain from several locations within the barrel. Once national survey samples have been taken, the elevator may empty the barrels.

If the barrel sample method cannot be used, wheat should instead be run out of the elevator's storage/transfer bins and collected from the belt using disposable 16-ounce plastic cups, until a 4-pound sample has been accumulated (including 250 grams more, for the reference sub-sample). Mechanical samplers (diverters) should be used, if available, for collecting samples from a belt. If samples are to be collected by this method, they should be taken from <u>each</u> available bin in the elevator (when possible). The collector should ask the elevator to run grain from the first bin for a short time, taking a sample directly from the belt or other accessible point. The process should be repeated for the next bins in succession until complete. All of the bin samples should then be combined, mixed, and the official composite 4-pound sample taken.

Other possible sources of samples for the survey include research stations/labs (to which producers have submitted grain samples for other purposes, and excess is available), farm storage bins, mills, trucks/combines, and rail cars. When collecting grain from these sources, every effort should be made to draw as representative a composite sample as possible. Questions about sampling procedures, methods, and sources may be directed to the KB national program managers (Table 2).

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# Sample Preparation and Processing

## **Processing Laboratories**

The USDA-APHIS-PPQ National KB Sample Processing Laboratory is located in Phoenix, Arizona. Samples are processed as they arrive, in the order they are received. Test results are reported to the submitting state and the APHIS KB program managers, usually within one to two weeks. The PPQ standard for analysis of grain samples is bunted kernel examination (FR 2004; NAPPO 2016).

States may opt to screen and analyze their own samples at a different lab. However, the screening standard that must be used is the bunted kernel examination, and all facilities must be approved by APHIS prior to analyzing KB samples (Table 1). PPQ does not provide resources to state labs for processing samples, except in those states with regulated areas.

#### Packaging of Samples

Place each grain sample in a new heavy-duty paper bag. Double bag the samples if heavy-duty bags are not available. Roll down the top of the bag to squeeze out as much air as possible, then securely tape the bag shut with strong packing tape (no staples). Mark the sample number on the paper bag. Place each paper-bagged sample into its own, secondary 6-mil plastic bag, along with a completed Sample Information Form (Appendix B), and then seal the plastic bag with a zip tie. These packing materials are available from PPQ as the KB Sample Survey Kit and can be ordered from IPHIS (Appendix C). If the PPQ Kit is not being used, then all materials should be of similar type and quality. Appendix C summarizes these instructions with pictures.

Samples should be shipped in a sturdy, crush-resistant box. Do not use soft mail or envelope bags, which can readily tear or puncture in transit. Limit the weight of each shipping box to 36 pounds (9 samples) or less.

#### Shipping of Samples

For states that use the PPQ Phoenix, AZ laboratory to analyze their samples, the *samples* should be shipped as soon as possible after collection using a 2-day delivery service to the address below:

USDA-APHIS-PPQ Becky Van Cleave 3640 E. Wier Avenue Phoenix, AZ 85040 (602) 431-3216 becky.j.van.cleave@usda.gov

Samples should not be collected and held for shipment all at the end of the season. Doing so impedes the seasonal workflow of the lab and risks unnecessarily delaying the detection of KB-positive samples. Lab analysis and reporting will occur in a timely

manner, generally within one to two weeks, so results will be available before potentially contaminated source grain can move farther down the marketing channel.

It is highly recommended to write the permit number of the PPQ Phoenix, AZ lab on a sheet of paper inside the shipping box, or on the outside of the box near the shipping label, or to include a copy of the permit itself with the shipment. This could help avoid delays with interstate shipments. The lab's current permit number is **P526P-20-03925** (expires 10-07-23). A shipping account is also available for use by cooperators and federal personnel when sending National KB Survey samples to the PPQ Phoenix, AZ lab. For further information, please contact the AZ lab directly (Table 1).

Table 1. Approved KB Sample Processing Laboratories.

State	Location	Contact Information
Arizona	USDA-APHIS-PPQ 3640 E. Wier Avenue Phoenix, AZ 85040	Becky Van Cleave Telephone: (602) 431-3216 Email: becky.j.van.cleave@usda.gov
California	California Department of Food & Agriculture Plant Pest Diagnostics Center 3294 Meadowview Road Sacramento, CA 95832	For current lab contact information, consult the applicable APHIS-PPQ State Plant Health Director
Delaware	University of Delaware Cooperative Extension Plant Diagnostic Lab 151 Townsend Hall Newark, DE 19716	For current lab contact information, consult the applicable APHIS-PPQ State Plant Health Director
Oklahoma	Oklahoma State University Entomology & Plant Pathology Department 315 Noble Research Center Stillwater, OK 74078	For current lab contact information, consult the applicable APHIS-PPQ State Plant Health Director

# Sample Analysis and Reporting

National survey samples submitted to the PPQ Phoenix, AZ lab are analyzed using an optical seed sorting machine. The high-speed optical sorter removes suspect bunted kernels from samples, can process a 4-pound sample in less than a minute, and typically will reduce the number of kernels that need to be examined to about 8% of the original sample. All suspect kernels are then visually examined by trained personnel.

APHIS approved laboratories may also use grain inspection machines and visual examinations by trained personnel to analyze samples for bunted kernels.

When a state's sampling is done for the year and its final samples are being submitted, the State KB Coordinator will notify the receiving laboratory that its season is over. The laboratory will in turn issue a summary report for that state within 10 days of the last samples received. A copy of this report should also be e-mailed to the <a href="State Plant">State Plant</a> Health Director (SPHD) (if different from the State Coordinator) and to the PPQ KB national program managers (Table 2).

#### **Negative Sample Reporting**

The laboratory conducting the sample analysis will report negative sample findings to the State KB Coordinator of the submitting state. The report should be sent by e-mail as soon as available but no more than one week after completion of the analysis. The <a href="State Plant">State Plant</a> Health Director (SPHD), if different from the State Coordinator, should also receive a copy of the report, along with the PPQ KB national program managers (Table 2).

## Preparation, Processing, and Confirmation of Suspect Positive Samples

When a suspect positive sample is detected at the initial screening lab, it must be confirmed by the PPQ National Specialist in Mycology. Procedures for preparing a bunted kernel specimen and microscope slide with teliospores are outlined in Appendix D of the <u>USDA Karnal Bunt Manual</u>.

Slides and specimens should be packed carefully in small cardboard boxes, so they do not break during shipping. The small specimen boxes should be placed within a larger, crush-resistant box with ample packing material. Copies of all relevant specimen documentation must be included inside the shipment.

When a suspect KB sample is submitted for diagnostic confirmation, the submitter should immediately notify all the following:

- The State Plant Health Director (SPHD)
- The State KB Coordinator in the state from which the suspect bunted kernel originated
- The PPQ Domestic Diagnostic Coordinator (PPQ.Domestic.Diagnostic.Coordinator@usda.gov)
- PPQ's National Operations Manager and National Policy Manager (Table 2).

For submitters with access to PPQ's Agricultural Risk Management (ARM) system, a Diagnostic Request (DR) should be created, and specimens should be routed according to the instructions on the DR. Submitters without access to ARM should contact their SPHD and prepare <a href="PPQ Form 391">PPQ Form 391</a>. Further guidance is available on the APHIS website here: <a href="Request Official Confirmation of Preliminary Pest Identifications of Domestic Samples">Pest Identifications of Domestic Samples</a>.

#### **Notification and Disposal of Positive Samples**

PPQ will notify the SPRO and the SPHD by email when the identification is final. If the sample is positive, any associated sub-samples retained at screening labs, or retained at

the office of origin, must be disposed of, when no longer otherwise needed, using an approved method (page 5).

# **National Program Contacts**

**Table 2. National KB Program Contacts** 

Role	Name, Address	Phone, Email
National Policy Manager	Lynn Evans-Goldner USDA-APHIS-PPQ 4700 River Road, Unit 160 Riverdale, MD 20737	Phone: (301) 851-2286 Email: lynn.evans-goldner@usda.gov
National Operations Manager	Alec Ormsby USDA-APHIS-PPQ 2150 Centre Ave Building B, 3E10 Fort Collins, CO 80526	Phone: (970) 494-7521 Email: alec.ormsby@usda.gov

# **Data Management**

State Survey Coordinators or their designees should enter all national KB survey data into the National Agricultural Pest Information System (NAPIS) database. In addition to putting the data into NAPIS, survey coordinators may choose to record the results in the "Integrated Plant Health Information System" (IPHIS) database as well, but that step is not required.

States are responsible for their own data entry. Staff at the PPQ Phoenix laboratory cannot perform that task on their behalf. Each state should complete its NAPIS data entry as follows (unless otherwise specified within a cooperative agreement):

- First record for the state and/or county within 48 hours of PPQ confirmation.
- All other records, including positive and negative survey data, within two weeks of receipt.

# Type of Data to Enter

Survey data entered should capture:

- Four-pound samples that represent a selected county with 1,000,000 bushels of production or more. (A 4-pound sample should be taken for every 1,000,000 bushels: see the guidance on sampling procedures above.)
- Four-pound samples that represent multiple counties with a combined total of 1,000,000 bushels of production or more, in cases where each individual county produces less than 1,000,000 bushels on its own.

#### **How to Enter Data**

Data for KB national survey samples will be entered based on county. Three possible scenarios are:

- 1. Sample ST-100 was drawn from one county in **one** location; therefore, it is entered once.
- 2. Sample ST-101 was drawn from one county with several locations. This sample is a composite; however, it should be entered once.
- 3. Sample ST-102 was drawn from four counties from several locations. This combined sample would be entered **four times** under each individual county, so that each county receives credit for participating in the survey. With each entry, indicate in the remarks section that the sample represents a composite, and list the names of the other counties.

#### **NAPIS Codes and Definitions**

Selected codes for use with the NAPIS Karnal Bunt survey template are provided below, along with their definitions.

Not all of the template categories are shown in these guidelines: only the ones where information on available options may help with the correct and accurate entry of records. Refer to the on-line NAPIS/CAPS "data definitions" page, under the NAPIS "data entry" tab, for additional details, if needed.

Table 3. NAPIS template category for "Data Source"

Code Number	Definition
11	USDA-APHIS
13	state department of agriculture
14	university/extension
16	joint state/federal
18	state experiment station
20	state agriculture department of forestry
46	county agriculture department
99	unspecified/other

Table 4. NAPIS template category for "Site"

Code Number	Definition
24000	grain, general
24007	common wheat, <i>Triticum aestivum</i>
24011	triticale, <i>Triticosecale rimpaui</i>
24016	durum wheat, <i>Triticum turgidum</i>
24018	winter wheat, <i>Triticum aestivum</i>

24019	spring wheat, <i>Triticum aestivum</i>
28065	wheat, <i>Triticum</i> spp.

# Table 5. NAPIS template category for "Crop Life Stage"

Code Number	Definition
10	mature
13	post-harvest

# Table 6. NAPIS template category for "Crop Situation"

Code Number	Definition
24000	stored grains
28037	field or agronomic
29013	field inspection for export
29015	research center / experiment station
61012	farm seed/grain storage
71011	grain elevator
77004	commercial storage / warehouse

# Table 7. NAPIS template category for "Funding Source"

Code Number	Definition
4	state
6	other

# Table 8. NAPIS template category for "Survey Name"

Code Number	Definition
93	program: Karnal bunt

# Table 9. NAPIS template category for "Pest"

Code Number	Definition
FLBBNBF	Karnal bunt, <i>Tilletia indica</i>

# Table 10. NAPIS template category for "Pest Life Stage"

Code Number	Definition
P1	pathogens – asexual

# Table 11. NAPIS template category for "Survey Method"

Code Number	Definition
3011	general tissue sample

Table 12. NAPIS template category for "Sample Description"

Code Number	Definition
23	X pounds of grain from each of Y locations

Table 13. NAPIS template category for "Descriptor Units"

Code Number	Definition
373	samples

Table 14. NAPIS template category for "Diagnostic Lab"

Code Number	Definition
0816	Plant Pest Diagnostics Center, CDFA, Sacramento, CA
0830	Plant Diagnostic Clinic, U of Delaware, Newark, DE
1403	Karnal Bunt Processing Lab, APHIS-PPQ, Phoenix, AZ
3004	Plant Pathogen Confirmatory Diagnostics Laboratory
	(PPCDL), APHIS-PPQ, Laurel, MD
4602	Wheat Pathology Lab, Oklahoma State U, Stillwater, OK

[*Note*: All positive samples/records must show Lab 3004. Negative samples/records may show any of the listed labs, depending on where the final negative determination was made.]

Table 15. NAPIS template category for "Confirmation Method"

Code Number	Definition
9000	light microscope
9020	optical scanning

[Note 1: Code 9000 should be entered for all positive samples/records; it is also an option for use with negative samples/records.]

[Note 2: Code 9020 should be entered for negative samples/records only.]

#### **Technical Assistance**

For technical assistance with NAPIS, contact NAPIS User Support: <a href="https://napis.ceris.purdue.edu/support">https://napis.ceris.purdue.edu/support</a>.

For technical assistance with IPHIS, contact: **David Kowalski**, PPQ Data Steward, Phone: 970-494-7510, Email: david.g.kowalski@usda.gov.

#### References

FAS GATS. 2020. USDA Foreign Agricultural Service, Global Agricultural Trade System website.

FR. 2004. *Federal Register* Vol. 69, No. 35, Monday, February 23, 2004. Karnal Bunt; Revision of Domestic Regulations, Final rule.

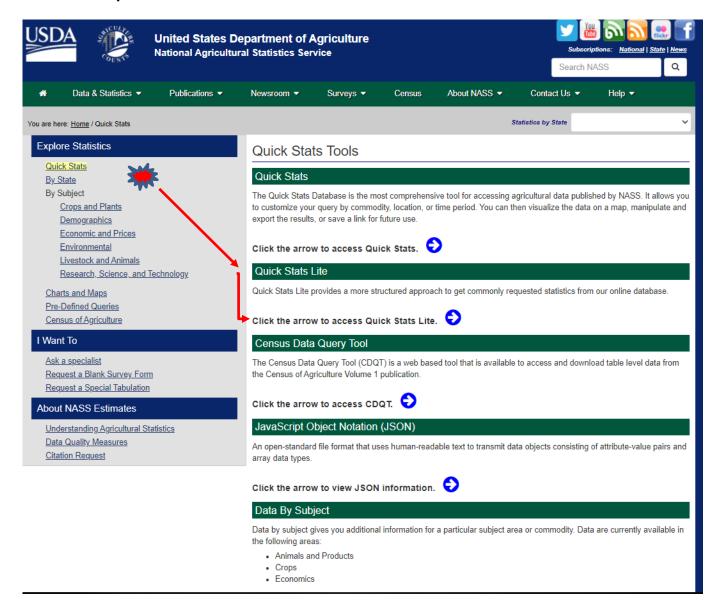
NAPPO. 2016. North American Plant Protection Organization. Regional Standards for Phytosanitary Measures (RSPM). RSPM 13: Guidelines to Establish, Maintain and Verify Karnal Bunt Pest Free Areas in North America.

USDA NASS. 2020. USDA National Agricultural Statistics Service website.

#### APPENDIX A - NASS Data Instructions

- 1) Begin at the "Quick Stats" page of the NASS website: <a href="http://www.nass.usda.gov/Quick Stats/index.php">http://www.nass.usda.gov/Quick Stats/index.php</a>.
- 2) Select the "Quick Stats Lite" option, as shown in the screenshot below.

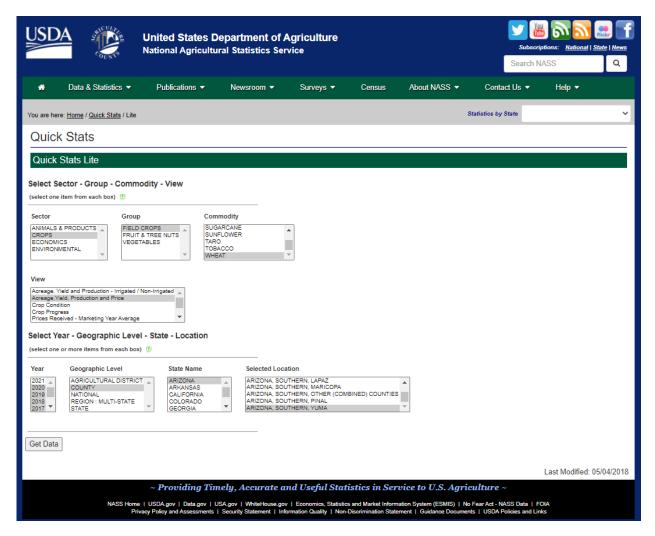
# Appendix A Job Aid 1. Screenshot of NASS Quick Stats Tools, Quick Stats Lite Option



3) Follow the prompts to obtain the wheat production by county. See the example in the next screenshot. Choose the 5 most recent years to calculate the average. Multiple years and counties can be selected by holding down the "Ctrl" or "Shift" button.

### Appendix A Job Aid 2. Screenshot of NASS Quick Stats Lite Option,

## Wheat Production by County



4) After selecting the "Get Data" button, an option will be available to download the results as a spreadsheet, for easier sorting and analysis.

<u>Note</u>: in some instances, a county may not show a published county yield for a given year due to confidentiality reasons or because it failed to meet USDA's publication standards (number of reports and/or coverage standards). In this case you will not be able to establish a true 5-year average from NASS.

#### **Appendix B – Sample Information Form**

NATIONAL KARNAL BUNT WHEAT GRAIN SURVEY SAMPLE INFORMATION FORM	
STATE SURVEY YEAR:	
NAME OF FACILITY WHERE SAMPLE WAS TAKEN	
CITY AND STATE OF FACILITY	
PRODUCTION COUNTY AND STATE	
TYPE OF WHEAT (HARD RED WINTER, DURUM WHEAT, TRITICALE, SOFT WHITE WINTER, Etc.) Please Specify Variety	
DATE SAMPLE WAS TAKEN	
SAMPLE TAKEN FROM (GRAIN ELEVATOR, TRUCK, MILL, RESEARCH, FARM STORAGE, RAIL CAR, Etc.)	
NAME, AND TELEPHONE # OF PERSON TAKING SAMPLE	
AGENCY OF PERSON TAKING SAMPLE (FEDERAL, STATE, UNIVERSITY)	
STATE SURVEY SAMPLE IDENTIFICATION NUMBER (EXAMPLE TX -100)	

Each state KB Survey Coordinator should establish and assign State Survey Sample Identification numbers for their state. The sample number should consist of the two-letter state abbreviation plus a sequential three-digit number beginning with 100 (e.g., TX-100). However, states that prefer a different numbering convention may use that instead. The original form should be completed on site as the sample is taken. Additional copies can be made for the State Plant Health Director and the State Plant Regulatory Official. The original form should accompany the sample to the lab that performs the analysis.

KB Sample Kits may be used for the National Survey (Figure 1). In IPHIS, order item #137, Karnal Bunt Survey Kit, consisting of one heavyweight brown paper bag, one 6-mil plastic bag, one zip tie and one 16-oz collection bottle. If other packaging material is used, it must be equivalent in quality/format to the above items.

# Appendix C Job Aid 1. Screenshot of IPHIS ordering system for Karnal Bunt Survey Kit





Figure 1. KB Sample Kit.

When submitting National KB Survey samples to the lab for processing, they **must be** four (4) pounds to meet the official standards (Figure 2).



Figure 2. Pan of wheat kernels on scale to confirm four-pound weight.

Using the KB Sample Kit, put the official sample into the sturdy paper bag (Figure 3). Label the paper bag, using a permanent marker, with the sample number from the Sample Information Form (Figure 4).



Figure 3. Four-pound sample of wheat in sturdy paper bag.



Figure 4. Sample bag labeled with number from Sample Information Form.

Use the collection jar to retain a 250-gram subsample from the excess wheat in your survey sample (Figure 5). Note the sample number on the jar for cross-reference. Maintain the subsample until the lab results are known.



Figure 5. Subsample of wheat kernels in collection jar.

The paper bags should be sealed with clear shipping package tape to prevent spillage (Figure 6). **Do not use staples**.



Figure 6. Labeled sample bag sealed with clear shipping package tape.

Place each four-pound grain sample into the 6-mil plastic bag provided in the KB Survey Sample Kit (Figure 7).

Place the completed "Sample Information Form" inside the plastic bag (Figure 7).

Seal the plastic bag with the enclosed zip tie (otherwise known as a cable tie) provided in the KB Survey Sample Kit (Figure 7).



Figure 7. Final sample bag and Sample Information Form in plastic bag, sealed with a zip tie.