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Tesis
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Barcelona, Junio 2013

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Facultat de Biologia – Departament de Biologia Animal
Programa de Doctorado "Biodiversidad"

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Memoria presentada por

Luigi Salis

para optar al título de
Doctor en la Universitat de Barcelona

El doctorando	**La directora**
Luigi Salis	**Marta Goula Goula**

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REGIONE AUTÒNOMA DE SARDIGNA
REGIONE AUTONOMA DELLA SARDEGNA

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"L'Amor che move il sole e l'altre stelle"

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ÍNDICE

INTRODUCCIÓN

LAS CHINCHES DE LOS CEREALES

Las chinches de los cereales, insectos que pertenecen a los géneros *Eurygaster* Laporte (1833) (Hemiptera, Heteroptera, Fam. Scutelleridae) y *Aelia* Fabricius (1803) (Hemiptera, Heteroptera, Fam. Pentatomidae), se alimentan de diversas gramíneas silvestres y cultivadas. El cultivo de trigo es uno de los afectados por estos insectos, que pueden producir un perjuicio tanto cuantitativo, es decir una pérdida de la cosecha, como cualitativo, con repercusión en la harina y sémola resultante.

Las chinches de los cereales están ampliamente distribuidas en varias zonas de Europa, Asia y el Norte de África (Paulian y Popov 1980). Se estima que más de 15 millones de hectáreas de cereales, principalmente trigo y cebada, están infestadas anualmente en Siria, Irak, Irán, Turquía, Afganistán y Líbano, así como en Asia Central y el Cáucaso, Bulgaria y Rumania (El Bouhssini et al. 2002).

Figura 1. Individuos de *Eurygaster* spp. (Foto L. Salis 2008)

El género *Eurygaster* (Fig.1) incluye 15 especies (Froeschner 1988a; Javahery et al. 2000; Göllner-Scheiding 2006), de los cuales sólo tres son plagas de los cereales: *Eurygaster integriceps* Puton (1881) *Eurygaster maura* (Linnaeus 1758) y *Eurygaster austriaca* (Schrank 1776) (Paulian y Popov 1980). *E. integriceps* se encuentra desde el sur de Europa hasta China, aunque no está presente en la Península Ibérica; *E. maura* está presente en Europa, África del Norte y Asia central y, por último, *E. austriaca* se extiende en toda la región euro-mediterránea hasta Asia central (Göllner-Scheiding 2006).

El género *Aelia* (Fig.2) incluye 16 especies (Froeschner 1988b; Derjanschi y Péricart 2005; Rider 2006), de las cuales *Aelia acuminata* (Linnaeus 1758) y *Aelia rostrata* Boheman (1852) son conocidas por ser importantes plagas de los cereales. Además, *Aelia germari* Küster (1852) y *Aelia klugii* Hahn (1833), pueden causar daño ocasionalmente (Paulian y Popov, 1980). Tanto *A. acuminata* y *A. klugii* son especies paleárticas, y *A. rostrata* está presente en la región euromediterránea, llegando hasta la India, mientras que *A. germari* sólo se encuentra en la cuenca del Mediterráneo (Rider 2006).

Figura 2. Individuos de *Aelia* spp. (Foto M. Pujol 2006)

Las chinches de los cereales son generalmente univoltinas (Javahery 1996). En la primavera, los adultos que han hibernado copulan y depositan sus huevos en los campos de cereales. Después de pasar por cinco etapas ninfales, aparece la nueva generación de adultos (Voegele 1996).

En otoño e invierno los adultos entran en diapausa después de migrar a través de distancias considerables o dispersarse a nivel local a los sitios de hibernación (Brown 1965; Javahery 1996; Voegele 1996). *Aelia* spp. y *Eurygaster* spp. tienen una diapausa obligada en toda su área geográfica que está influenciada tanto por el fotoperiodo como por la temperatura (Javahery 1996).

Las chinches de los cereales hibernan como adultos en varios refugios, como piedras, hojas secas y matas de hierba (Voegele 1996). En primavera, cuando aumentan las temperaturas, estos adultos se trasladan a los campos de cereales para alimentarse y copular, y mueren poco después de terminar la ovoposición.

La alimentación es una prioridad esencial en la primavera para el primer apareamiento y ovoposición para ambos sexos (Javahery 1996). Algunas especies de *Eurygaster* y *Aelia* son fuertemente migratorias desplazándose más de 20 km, mientras que otras son sedentarias, o sólo están sujetos a una dispersión muy pequeña. La invasión o no por *Eurygaster* spp. y *Aelia* spp. de las áreas que parecen ser ecológicamente adecuadas, puede explicarse de acuerdo con la dirección del viento (Brown 1965). El daño a la cosecha es proporcional a la densidad de las chinches del trigo que está directamente relacionada con el éxito de la hibernación. Este éxito a su vez depende de la acumulación de reservas de grasa antes de la hibernación (Donkstoff 1996). Los cambios en la densidad de población y los brotes de esta plaga están determinados en gran medida por factores abióticos y bióticos externos. Las condiciones climáticas, especialmente la temperatura y las precipitaciones, juegan un papel importante en la dinámica poblacional de las chinches de los cereales. Las precipitaciones continuas retrasan la actividad de estos insectos, y los largos períodos con alta humedad en los sitios de hibernación causan una elevada mortalidad (Javahery 1996). Entre los enemigos naturales encontrados los más importantes pertenecen a los grupos Hymenoptera, Diptera y Fungi (Voegele 1996) que contribuyen a la regulación de las poblaciones. Los márgenes de los campos son la principal fuente de muchos enemigos naturales de esta plaga (Tshernyshev et al. 2010).

La importancia económica de las chinches de los cereales se debe a las pérdidas de cosecha y/o a la pérdida de calidad del trigo (Kinaci y Kinaci 2004), o de la sémola (Ozderen et al. 2008; Köksel et al. 2009; Salis et al. 2010) o de la harina (Hariri et al. 2000; Sivri et al. 1999, 2004; Aja et al. 2004; Vaccino et al. 2006), y también en gran medida a la reducción en el porcentaje de germinación del trigo (Bin et al. 2006).

Las chinches causan los daños mencionados por efecto de su actividad alimenticia tanto en la fase adulta como ninfal, cuando insertan sus partes bucales perforadoras-chupadoras en los granos y extraen las sustancias en su interior. Con el fin de facilitar la succión de los elementos nutritivos del endosperma, los granos son digeridos externamente mediante la inyección de saliva rica en proteasas (Sivri et al. 1998; Konarev et al. 2011) y amilasas (Kazzazi et al. 2005). Si el grano ha sido picado durante las primeras fases de desarrollo la superficie se presenta deformada (Critchley 1998; Hariri et al. 2000). Después de la picada alimenticia de las chinches, normalmente, la maduración del grano continúa. Si el grano ha sido picado cuando ya estaba maduro (Fig.3), aparece una mancha opaca blanquecina, debida al efecto visual del reflejo del aire que ha quedado en el interior del grano; en algunas ocasiones, en el punto de la picadura se reconoce un punto negro.

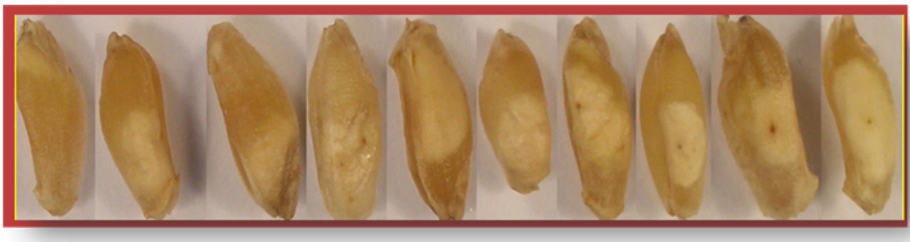

Figura 3. Granos de trigo duro picados por las chinches de los cereales
(Foto S. Ojeda e Y. Robles 2008)

En Italia, aunque el problema de las chinches de los cereales no se ha tomado en cuenta de la forma que le correspondería, la presencia y los daños causados por estos insectos se han constatado en numerosas ocasiones. Malenotti (1931) reportó una fuerte infestación en 1931 de *A. acuminata* en la provincia de Verona, donde también se hallaron individuos de *E. maura* y *Eurygaster hottentota* (Fabricius 1775). En 1932-1933 se registró una fuerte infestación de *A. rostrata* en las provincias de Verona, Mantua y Brescia y también se encontraron individuos de *E. maura* (Malenotti 1933). Se señalaron infestaciones menos importantes en el sur de Italia, sobre todo en la región de Puglia (Genduso y Di Martino 1974). En Sicilia, en 1973/74 y 1975 se describieron fuertes infestaciones de *A. rostrata*, junto con la presencia de *E. maura* y *E. austriaca* (Genduso 1977). En 1998/99 se registraron en Piamonte, en localidades de las provincias de Alessandria y Asti, ataques significativos de *E. maura*, y en menor medida también de *E. austriaca* y *Aelia* spp. (Tavella y Migliardi 2000). En el año 2000, se anotó en Sicilia la presencia de *A. rostrata* (Spina 2000). En 2005 ocurrió una fuerte infestación de *Eurygaster* spp. sobre trigo blando en Italia central (Val di Chiana, Toscana) y requirió un tratamiento con insecticida (Bin et al. 2006).

El efecto perjudicial de la actividad alimenticia de las chinches de los cereales ha sido estudiado principalmente en el trigo blando (*Triticum aestivum*) (Fogliazza et al. 2006, Hariri et al. 2000; Sivri et al. 1998, 1999, 2004; Vaccino et al. 2006), pero no en otras especies del género *Triticum*. Existen varios estudios sobre el porcentaje mínimo necesario de granos de trigo dañados por los chinches de los cereales para afectar de manera significativa la calidad panificadora. Porcentajes del 3-5% de granos dañados tienen ya efectos perjudiciales, y estos efectos aumentan dramáticamente para valores superiores a 10% (Karababa y Ozan 1998; Hariri et al. 2000). En Europa, un nivel entre 1-10% de granos dañados ya determina una disminución de la calidad panificadora (Karababa y Ozan 1998).

EL TRIGO DURO

El trigo (*Triticum* spp.) es un cereal muy versátil, se cultiva en todos los continentes y existe una amplia gama de cultivares adaptados a condiciones locales. El cultivo de trigo es uno de los tres más importantes en relación al área cultivada (aproximadamente 215 millones de hectáreas) y a la cosecha total (aproximadamente 625 millones de toneladas) (FAO 2013). Las propiedades únicas de la harina de trigo y de la masa permiten la producción de una gran variedad de productos, entre ellos varios tipos de pan, pasta, pasteles y galletas. El trigo duro (*Triticum turgidum* L. var. *durum*) sólo representa alrededor del 6-8% de la producción total de trigo (Troccoli et al. 1998), ya que la mayor parte de la producción de trigo está representada por el trigo blando (*Triticum aestivum* L.).

La producción mundial de trigo duro es de unos 35 millones de toneladas e Italia es uno de los productores más importantes, generando alrededor del 15% de la producción mundial (Taylor y Woo 2011) y el 60% de la producción en la Unión Europea. En 2011, Italia produjo aproximadamente unos 3,9 millones de toneladas de trigo duro (ISTAT 2013). El trigo duro constituye alrededor del 70% de la superficie total cultivada con trigo en Italia. En Cerdeña, isla italiana donde se ha llevado a cabo gran parte del estudio de esta tesis, el cultivo del trigo duro es el más extendido. Se cultiva en cerca de 84.000 hectáreas, con una producción media en los últimos años de alrededor de 134.000 toneladas por año (ISTAT 2013). El trigo duro es un cultivo muy antiguo en la cuenca del Mediterráneo y se utiliza principalmente para la fabricación de pasta incluyendo cuscús y bulgur, y además tradicionalmente para la producción de panes típicos, como por ejemplo en algunas regiones italianas (Quaglia 1988). En Cerdeña, el trigo duro se utiliza tanto para la confección de la pasta como para la producción de pan, y este último tiene un especial interés tanto desde una perspectiva económica como desde un punto de vista cultural (Dexter y Marchylo 2000). En Cerdeña las dos formas más comunes de panes típicos son la “Spianata" (Fig. 4) y el “Carasau” (Fig. 5). El primero es un pan suave de doble capa, similar al pan de pita (se consume ampliamente en el Mediterráneo oriental), mientras que el "Carasau" es de una capa delgada y crujiente, y sigue siendo comestible durante varios meses sin ninguna necesidad de conservante añadido (Fois et al. 2011).

Figura 4. Foto pan "Spianata". (Foto R. Brotzu 2008)

Figura 5. Foto pan "Carasau". (Foto E. Messina 2008)

LA PASTA

La pasta es un producto muy antiguo, conocido en la Roma antigua en el I siglo antes de Cristo, aunque hasta hoy sobrevive la leyenda publicada en el año 1929 por un periodista estadounidense según la cual el viajero veneciano Marco Polo volviendo de su viaje a Asia al final del siglo XIII trajo "spaghetti" a Italia (Agnesi 1996; Montanari 2008).

La pasta es un producto popular porque es versátil y su producción es relativamente fácil mezclando sémola y agua. La pasta se encuentra en una gran variedad de formas, se puede almacenar y conservar durante largos periodos, se puede transportar fácilmente y tiene una excelente calidad nutricional e higiénica (Feillet y Dexter 1996).

La producción mundial de pasta en el 2011 fue alrededor de 10,5 millones de toneladas, de las cuales 3,3 millones de toneladas procedieron de Italia, el mayor productor mundial (IPO 2012). En cuanto al consumo anual, el primer país es Estados Unidos con 2,7 millones de toneladas seguido por Italia con alrededor de 1,6 millones de toneladas, mientras que en España se consumen alrededor de 235000 toneladas (IPO 2012). Considerando el consumo anual per cápita, destaca en primer lugar Italia, con 26 kg, seguida de Venezuela, con 12,3 kg, mientras que en España el consumo anual per cápita es de 5 kg (IPO, 2012).

La sémola de trigo duro es la materia prima preferida para elaborar pasta alimenticia de calidad superior, que se produce extrusionando una masa firme de sémola y agua (Feillet y Dexter 1996). La calidad tecnológica del trigo duro, depende del rendimiento en sémola y de la calidad de sémola, que es la capacidad de la sémola de ser transformada en pasta que cumple con los requisitos de los consumidores habituales (Cubadda 1988).

Las proteínas tienen una gran importancia en la determinación de las propiedades funcionales de la harina y de la sémola, en particular las proteínas del gluten, de modo que está universalmente reconocido que el contenido de proteínas es el factor principal que determina la calidad de la pasta y que la fuerza del gluten es un factor secundario importante (D'Egidio et al. 1990;

Novaro et al. 1993; Feillet y Dexter 1996). Por esa razón la mayoría de los fabricantes de pastas exigen un contenido de proteína mínimo en la sémola (Feillet y Dexter 1996).

El gluten del trigo consiste en más de 50 componentes proteicos, que tradicionalmente han sido clasificados en dos grupos, las gliadinas y las gluteninas (Shewry et al. 1997). Las gliadinas son solubles en disoluciones acuosas de alcoholes y están presentes como proteínas monoméricas que no tienen enlaces disulfuro inter cadenas y sí tienen enlaces disulfuro intra cadena. Las gluteninas son insolubles en disoluciones acuosas de alcoholes y están formadas por polímeros de subunidades proteicas enlazadas con enlaces disulfuros (Shewry et al. 1997). Ambas fracciones contribuyen de manera importante a las propiedades reológicas de la masa. Las gliadinas confieren sobre todo viscosidad y extensibilidad a la masa, mientras las gluteninas dan fuerza y elasticidad (Wieser 2007).

Las características de textura de la pasta juegan un papel esencial en la determinación de la aceptación final por los consumidores. En los países tradicionalmente consumidores de pasta como Italia, los atributos de textura son prácticamente los únicos que se consideran, y en segundo término también el color. Las características de textura, directamente relacionadas con la resistencia a la cocción, son importantes para el consumo en general, donde la pasta debe mantener su textura no sólo con el tiempo normal de cocción sino también con sobre cocción (D'Egidio 1996).

La calidad de la pasta se evalúa preferentemente con "spaghetti", que al ser cilíndricos, tienen la mejor forma geométrica para dar la mejor indicación de la calidad intrínseca de la sémola de trigo duro. Las características de textura de los "spaghetti" cocidos pueden ser medidas con una evaluación sensorial o con métodos objetivos (químicos o instrumentales). El tiempo de cocción, la cantidad y la calidad de agua utilizada y el tiempo de escurrimiento de la pasta son factores a tener en cuenta en la evaluación de la pasta.

La evaluación sensorial toma en consideración tres parámetros: pegajosidad, firmeza y la tendencia a agregarse ("bulkiness"). Estos parámetros se evalúan visualmente, manualmente y masticando la pasta. A cada parámetro se le asigna una puntuación de 0 a 100. La puntuación total se calcula con la media de los tres parámetros, de modo que a mayor puntuación, mejor calidad de la pasta. Generalmente se necesitan por lo menos de tres evaluadores bien entrenados para hacer la evaluación sensorial (D'Egidio 1996).

Tabla 1. Parámetros de evaluación sensorial de la pasta y puntación (Adaptada desde D'Egidio 1996)

EVALUACIÓN SENSORIAL DE LOS PARAMETROS TEXTURALES DE LOS "SPAGHETTI" COCIDOS			
PEGAJOSIDAD	**FIRMEZA**	**TENDENCIA A AGREGARSE**	**PUNTUACIÓN**
MUY ALTA	MUY ALTA	AUSENTE	<20
ALTA	ALTA	ESCASA	40
ESCASA	ESCASA	SUFICIENTE	60
CASI AUSENTE	CASI AUSENTE	BUENA	80
AUSENTE	AUSENTE	MUY BUENA	100

Entre los métodos objetivos para la evaluación de la calidad de cocción de la pasta están la absorción de agua durante la cocción, la pérdida de material durante la cocción y la determinación de la materia orgánica total (TOM). Considerando la absorción de agua durante la cocción, cuanta más agua absorbe la pasta menor será la calidad. La pérdida de material orgánico durante la cocción, en particular almidón, está relacionado negativamente con la calidad de la pasta (D'Egidio 1996).

La determinación del material liberado en el agua de lavado de la pasta después de la cocción, indicado como TOM del inglés "Total Organic Matter", determina la cantidad de material que estaba adherido en la superficie de la pasta, sobre todo almidón, responsable de la pegajosidad de la misma. A valores de TOM más altos corresponden una calidad más baja de la pasta. El TOM se basa en un método químico y tiene una alta correlación con la evaluación sensorial (D'Egidio 1996).

OBJETIVOS

OBJETIVOS

LAS CHINCHES DE LOS CEREALES, EL TRIGO DURO Y LA PASTA

No existían estudios sobre la presencia de chinches en el trigo duro en Italia, incluida la isla de Cerdeña. Según los niveles de infestación de las chinches de los cereales habrá un porcentaje más o menos alto de granos dañados por la actividad alimenticia de las chinches. Esta actividad implica la inyección de proteasas en los granos de trigo. Las proteasas atacan las proteínas, que son las que tienen mayor influencia en la calidad de la sémola y la pasta producida con el trigo duro. En este trabajo de tesis, se estudia la presencia de las chinches de los cereales en el cultivo de trigo duro de Cerdeña, así como la influencia de esta plaga en la fracción proteica del trigo duro, particularmente en las gliadinas y gluteninas, y en el comportamiento de la sémola y la pasta derivadas de trigo duro con distintos porcentajes de granos dañados por las chinches de los cereales.

OBJETIVO PRINCIPAL

El objetivo principal de este trabajo de tesis fue el estudio de los efectos de las chinches de los cereales en el trigo duro (*Triticum turgidum* L. var *durum*) a diferentes niveles: en el campo, a nivel macromelocular (proteínas) y en el producto final (sémola y pasta).

OBJETIVOS ESPECÍFICOS

Los objetivos específicos se expresan para cada uno de los capítulos del cuerpo de la tesis:

- Determinar las especies, la distribución y la densidad de las chinches de los cereales presentes en los campos de trigo duro de Cerdeña con el fin de conocer la incidencia de la plaga en la isla.
(Capítulo 1)

- Investigar los efectos de las chinches de los cereales a nivel macromolecular sobre las proteínas del gluten, gliadinas y gluteninas.
(Capítulo 2)

- Evaluar los efectos de las chinches de los cereales en los granos, en la sémola y la calidad de cocción de la pasta obtenida con trigo duro dañado (Capítulo 3)

INFORME DE LA DIRECTORA DE TESIS

INFORME DE LA DIRECTORA DE TESIS

Como directora de la tesis doctoral titulada “Las chinches de los cereales (*Eurygaster* Fam. Scutelleridae; *Aelia* Fam. Pentatomidae): importancia y efectos en el trigo duro (*Triticum turgidum* var. *durum* L.), especialmente en Cerdeña (Italia)” certifico que el trabajo presentado ha sido llevado a cabo en su totalidad por Luigi Salis.

Los trabajos presentados en esta tesis han sido publicados o están en preparación para su envío a revistas científicas internacionales. A continuación se detallan los artículos con su factor de impacto (Thomson Scientific Web of Science) y las contribuciones de los autores a cada artículo:

CAPÍTULO 1

Population density and distribution of wheat bugs (genera *Eurygaster* and *Aelia*) infesting durum wheat in Sardinia (Italy)

L. Salis, M. Goula, J. Izquierdo and E. Gordún

Journal of Insect Science 2013 13(50), 1-15
Factor de Impacto: 0.947 – Categoría "Entomología" (2º cuartil)

Concepción y diseño de los experimentos: **L. Salis**, E. Gordún, M. Goula; Realización de la parte experimental: **L. Salis**, M. Goula; Análisis de datos: **L. Salis**, E. Gordún, J. Izquierdo; Redacción del artículo: **L. Salis**; Revisión del artículo: E. Gordún, M. Goula, J. Izquierdo.

CAPÍTULO 2

Prolamin proteins alteration in durum wheat by species of the genus *Eurygaster* and *Aelia* (Insecta, Hemiptera)

L. Salis, M. Goula, J. Valero and E. Gordún

Spanish Journal of Agricultural Research 2010 8(1), 82-90
Factor de Impacto: 0.615 – Categoría "Agricultura/Multidisciplinario" (2º cuartil)

Concepción y diseño de los experimentos: **L. Salis**, E. Gordún; Realización de la parte experimental: **L. Salis**, E. Gordún; Análisis de datos: **L. Salis**, E. Gordún, J. Valero; Redacción del artículo: **L. Salis**; Revisión del artículo: E. Gordún, M. Goula, J. Valero.

CAPÍTULO 3

Wheat bugs effects on durum wheat (*T. turgidum* var. *durum*): from grain to pasta

L. Salis, M.G. D'Egidio, F. Giunta, M. Goula, J. Valero and E. Gordún

Food Chemistry (en preparación)
Factor de Impacto: 3.655 – Categoría "Ciencia y tecnología de alimentos"
(1er cuartil)

Concepción y diseño de los experimentos: **L. Salis**, M.G. D'Egidio, F. Giunta E. Gordún; Realización de los experimentos: **L. Salis**; Análisis de datos: **L. Salis**, M.G. D'Egidio, E. Gordún, J. Valero; Redacción del artículo: **L. Salis**; Revisión del artículo: M.G. D'Egidio, F. Giunta E. Gordún, M. Goula, J. Valero.

Certifico además que ninguno de estos trabajos científicos ha sido utilizado en otras tesis doctorales.

Directora

Dra. Marta Goula Goula

Profesora Titular
Departament de Biologia Animal
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CAPÍTULO 1

Densidad de población y distribución de las chinches de cereales que infestan el trigo duro de Cerdeña (Italia)

Resumen

El trigo es un cultivo muy importante en Italia y no está libre de la presencia de las chinches de los cereales pertenecientes a los géneros *Eurygaster* (Hemiptera: Scutellaridae) y *Aelia* (Hemiptera: Pentatomidae). Muchas infestaciones de esta plaga han sido reportadas en toda Italia, tanto en el pasado como recientemente. Este estudio se llevó a cabo en Cerdeña durante dos años (2007 y 2008). El objetivo de este estudio es determinar las especies y la distribución de las chinches de los cereales presentes en los campos de trigo duro de Cerdeña, y estimar su densidad de población con el fin de conocer la incidencia de la plaga en la isla. El muestreo se llevó a cabo dos veces al año (mayo y junio) en tres zonas, representativas de los cultivos de trigo duro en la isla. Se encontraron cuatro especies de insectos de trigo. La especie predominante fue *Eurygaster austriaca* (Schrank), seguida de *Aelia germari* (Kuster), *Eurygaster maura* L. y *Aelia acuminata* L. La densidad media de las chinches de los cereales fue baja (1,1 individuos/m^2), pero en ciertas áreas estaba por encima del umbral de daño (4 individuos/m^2). Por esta razón, la conclusión del estudio es que la plaga se debe supervisar con el fin de controlar los brotes y evitar su propagación, con el fin de mejorar la calidad de esta importante materia prima que es el trigo duro.

Journal Reference:

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Population density and distribution of wheat bugs infesting durum wheat in Sardinia, Italy

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ABSTRACT

Wheat is a very important crop in Italy, and is infested by wheat bugs belonging to the genera *Eurygaster* (Hemiptera: Scutellaridae) and *Aelia* (Hemiptera: Pentatomidae). Many wheat bug infestations have been reported in the north, south, and center of Italy, both in the past as well as recently. The present study was carried out in Sardinia, Italy, during two years (2007 and 2008). The objective of this study was to determine the species and distribution of wheat bugs in durum wheat fields in Sardinia, and to estimate their population density in order to know the incidence of the pest on the island. Sampling took place twice a year (May and June) in three zones, representative of durum wheat cropping in the island. Four species of wheat bugs were found; the predominant species was *Eurygaster austriaca* (Schrank), followed by *Aelia germari* (Kuster), *Eurygaster maura* L., and *Aelia acuminata* L. The average density of wheat bugs was low (1.1 individuals/m^2), but in certain areas it was above the damage threshold (4 individuals/m^2). For this reason, the conclusion of the study is that this pest should be monitored in order to control outbreaks and prevent their further spread.

INTRODUCTION

The names "sunn pest" and "wheat bug" refer to different species in the genera *Eurygaster* (Hemiptera: Scutellaridae) and *Aelia* (Hemiptera: Pentatomidae). Wheat bugs are widely distributed in various areas of Europe, Asia, and North Africa (Paulian and Popov 1980). An estimate of more than 15 million hectares of cereal, mainly wheat and barley, are infested annually in Syria, Iraq, Iran, Turkey, Afghanistan, and Lebanon, as well as in Central Asia and the Caucasus, Bulgaria, and Romania (El Bouhssini et al. 2002).

The genus *Eurygaster* includes 15 species (Froeschner 1988a; Javahery et al. 2000; Göllner-Scheiding 2006), of which only three are cereal pests, namely *Eurygaster integriceps* (Puton), *Eurygaster maura* L., and *Eurygaster austriaca* (Schrank) (Paulian and Popov 1980). *E. integriceps* is found from southern Europe up to China, although it is absent from the Iberian Peninsula; *E. maura* is present in Europe, North Africa, and central Asia; and finally, *E. austriaca* extends across the Euromediterranean region up to central Asia (Göllner-Scheiding 2006).

The genus *Aelia* includes 16 species (Froeschner 1988b; Derjanschi and Péricart 2005; Rider 2006), of which both *Aelia acuminata* L. and *Aelia rostrata* (Boheman) are known to be important cereal pests. In addition, *Aelia germari* (Kuster) and *Aelia klugii* (Hahn), among other *Aelia* species, can cause occasional damage (Paulian and Popov 1980). Both *A. acuminata* and *A. klugii* are Palaearctic species, and *A. rostrata* is present in the Euromediterranean region, extending eastwards up to India, whereas *A. germari* is found only in the Mediterranean basin (Rider 2006).

Wheat bug populations are generally univoltine, with the exception of certain *Aelia* species (Javahery 1996). In the spring, adults that have overwintered copulate and oviposit in the cereal fields, and the new generation of adult wheat bugs appears after going through five nymphal stages (Voegelé 1996). In autumn and winter, these adults undergo diapause after migrating over considerable distances or dispersing locally to overwintering sites (Brown 1965; Javahery 1996; Voegelé 1996). *Aelia* spp. and *Eurygaster* spp. undergo obligate diapause throughout their geographical range, and the diapause is influenced both by photoperiod and temperature (Javahery 1996). They hibernate as adults in various shelters including stones, dry leaves, and grass clumps (Voegelé 1996). All wheat bugs overwinter until temperatures rise in spring, at which time they move to cereal fields to feed and mate. The adults die soon after completing oviposition. Feeding in spring is essential prior to the first mating and oviposition for both sexes (Javahery 1996). Some *Eurygaster* and *Aelia* species are strongly migratory (> 20 km) while others are sedentary or only subject to very minor dispersion. Whether or not *Eurygaster* spp. and *Aelia* spp. invade areas that appear to be ecologically suitable may be explained according to wind direction (Brown 1965). Damage to the crop is proportional to the density of wheat bugs. Population density is directly related to hibernation success, which in turn depends on the accumulation of fat reserves prior to hibernation (Donkstoff 1996). Changes in population densities and outbreaks of these insects are largely determined by external abiotic and biotic factors. Climatic conditions, especially temperature and rainfall, play an important role in the population dynamics of wheat bugs. Continuous rainfall delays wheat bug activity, and long periods of high humidity in the overwintering sites cause mortality (Javahery 1996). Among the natural enemies observed, the most important belong to Hymenoptera, Diptera, and Fungi (Voegelé 1996), and they contribute to the regulation of wheat bug populations. Field margins are the main source of many natural enemies of this pest (Tshernyshev et al. 2010).

The economic importance of wheat bug damage is due to crop losses and/or quality loss of wheat (Kinaci and Kinaci 2004), semolina (Ozderen et al. 2008; Köksel et al. 2009; Salis et al. 2010), or flour (Hariri et al. 2000; Sivri et al. 1999, 2004; Aja et al. 2004; Vaccino et al. 2006; Werteker and Kramreither 2008). The feeding activity of wheat bugs also heavily affects the germination percentage of wheat (Bin et al. 2006). Both nymphs and adults of *Eurygaster* spp. and *Aelia* spp. cause a reduction of wheat quality when they insert their piercing-sucking mouthparts in the kernels and extract the substances within.

In order to facilitate the suction of the nutritional elements of the endosperm, the kernels are digested externally by injecting saliva rich in proteases (Sivri et al. 1998; Konarev et al. 2011) and amylases (Kazzazi et al. 2005). The detrimental effect of such proteases on baking quality is very high, even when only 3–5% of kernels are damaged, and dramatically increases for values higher than 10% (Karababa and Ozan 1998; Hariri et al. 2000).

In Italy, wheat is not free of wheat bugs. Malenotti (1931) reported a heavy infestation of *A. acuminata* in 1931 in the province of Verona, and *E. maura* and *Eurygaster hottentota* F. were also found. In 1932–1933, a heavy infestation of *A. rostrata* was recorded in the provinces of Verona, Mantova, and Brescia, and *E. maura* was also found (Malenotti 1933). Less important infestations have been registered in the south of Italy, particularly in the Puglia region (Genduso and Di Martino 1974). Severe infestations of *A. rostrata*, together with the presence of *E. maura* and *E. austriaca*, were registered in Sicily in 1973–1975 (Genduso 1977). In 1998–1999, significant attacks of *E. maura*, and to a lesser extent *E. austriaca* and *Aelia* spp., were reported in Piedmont and on localities in the provinces of Alessandria and Asti (Tavella and Migliardi 2000). In 2000, *A. rostrata* was recorded in Sicily (Spina 2000). In 2005, a heavy infestation of *Eurygaster* spp. on soft wheat occurred in central Italy (Val di Chiana, Toscana) and required an insecticide treatment (Bin et al. 2006).

Durum wheat (*Triticum turgidum* L. var *durum*) is one of the most important crops in Italy, a country that generates around 50% of the total production of durum wheat of the European Union, and around 15% of the world production (Sgroi and Fazio 2008). In 2008, Italy produced approximately 5.2 million tonnes of durum wheat (ISTAT 2010). Durum wheat constitutes ~70% of the total area cultivated with wheat in Italy. In Sardinia, durum wheat is the most widespread crop; it is grown on about 84,000 hectares, with an average production of about 134,000 tonnes per year (ISTAT 2010). Durum wheat is a very ancient crop in the Mediterranean basin and is used mainly to manufacture pasta, as well as for baking traditional types of bread (Quaglia 1988) with a particular interest both from an economic and a cultural point of view (Dexter and Marchylo 2000); the carasau bread, for example, made from durum wheat, is one of the most important products of the Sardinian bread making tradition (Dettori et al. 2002).

No studies on the distribution and density of wheat bugs have been carried out in Sardinia. Considering the importance of durum wheat in the Sardinian economy, a detailed knowledge of the species' distribution is required as a first step to develop sustainable management options for the improvement of the quality of wheat.

The aim of this study was to determine the species of wheat bugs present in the durum wheat fields of Sardinia, Italy, to explore their distribution in the island, and to estimate their population density in order to know the incidence of the pest on the island.

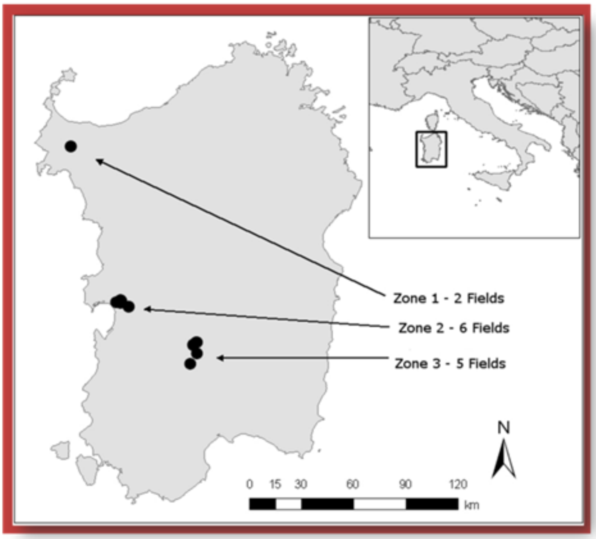


Figure 1. Zones and location of the fields sampled in Sardinia, Italy.

MATERIALS AND METHODS

The survey of wheat bugs was conducted during 2007 and 2008 in 13 durum wheat fields distributed in three different zones (Zone 1, Zone 2, and Zone 3), which were representative of durum wheat cropping in Sardinia

(Figure 1; Table 1). Fields were selected at random within each zone. The number of sampled fields in each zone was proportional to the cultivated area.

Table 1. Geographic coordinates, altitude, and areas of sampled fields and meteorological stations of each zone.

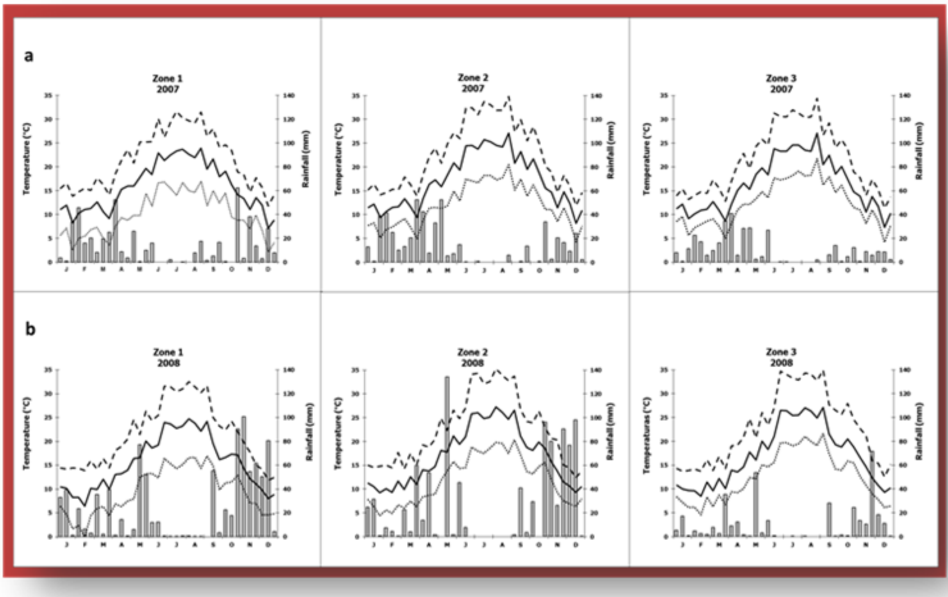
	Geographical coordinates		Altitude (m.a.s.l.)	Area (ha)
	Latitude	Longitude		
Zone 1				
Field 1	40°43'51.36"N	8°19'28.33"E	67m	7 ha
Field 2	40°43'41.92"N	8°19'20.52"E	70m	8 ha
Meteorological station (Olmedo-SS)	40º39'43''N	8º21'44''E	32m	
Zone 2				
Field 3	39°55'26.74"N	8°34'54.93"E	3m	8ha
Field 4	39°55'27.12"N	8°35'7.14"E	2m	5ha
Field 5	39°54'24.58"N	8°34'38.43"E	4m	1 ha
Field 6	39°53'29.98"N	8°37'40.60"E	8m	1.1 ha
Field 7	39°53'33.13"N	8°37'43.56"E	7m	3.3 ha
Field 8	39°54'8.22"N	8°33'10.32"E	1m	13 ha
Meteorological station (Milis - OR)	40º03'58''N	8º38'42''E	125m	
Zone 3				
Field 9	39°38'51.04"N	8°58'45.21"E	128m	7.5 ha
Field 10	39°42'4.19"N	8°58'53.30"E	185m	2.5 ha
Field 11	39°42'11.63"N	8°58'57.01"E	190m	2.3 ha
Field 12	39°41'50.40"N	8°57'2.34"E	181m	1.4 ha
Field 13	39°35'31.97"N	8°56'50.11"E	95m	5 ha
Meteorological station (Sardara - CA)	39º36'02''N	8º51'26''E	197m	

In the surveyed fields, neither pesticides nor fungicides were used, according to common agricultural practice in the region. In each field, insects were collected along six transects. Three transects covered the entire field edge, and the other three covered the interior of the field, following the protocol described by Pérez-Rodríguez et al. (2008). The field edge was considered to be the area between the border of field and two linear meters into the field, while the remaining part of the field was considered to be the interior. Along each transect, insects were collected in 15 regularly spaced sampling points. At each sampling point, an entomological sweep net with an opening of 0.17 m^2 was swept once over the cereal spikes in order to collect bugs. In other words, the total area sampled per transect was 2.55 m^2.

Considering six transects per field, a total of 90 points were sampled, equivalent to 15.3 m^2 sampled per field. Sampling took place twice a year, at the beginning of grain filling (1–10 May) to account for the initial population of bugs, and at grain maturation stage (10–20 June), just before harvest, to account for the bugs' final population. The insects collected were kept separately according to transect. Insects were preserved with ethyl acetate to keep them in good condition until identification of species and development stage (adults and nymphs) in the laboratory. The species were identified under the binocular microscope, and genitalia were studied when necessary. Species were identified according to Vidal (1949), Stichel (1957), Kis (1984), Tamanini (1988), and Ruiz et al. (2003).

Meteorological data of each zone were taken from the nearest meteorological stations to the sampled fields (Table 1). Maximum and minimum temperature and rainfall were recorded daily. The study area is characterized by a typical Mediterranean climate with long, hot, dry summers and short, mild, rainy winters. The climatic variables measured for the three zones in 2007 and 2008 are reported in Figure 2. The rainfall for Zone 1 and Zone 2 was very similar within the same year, around 500 mm in 2007 and around 900 mm in 2008. Zone 3 was much drier both years, with a rainfall of 350 mm. In 2007, Zone 2 registered the highest mean temperature (17.1º C) with respect to Zone 3 (16.3º C) and Zone 1 (15.8º C). In 2008, Zone 2 and Zone 3 registered the same mean temperature (16.9º C), while Zone 1 was the coldest zone both years with a mean temperature of 15.5º C.

Figure 2. Rainfall (bars), average (solid line), maximum (dashed line), and minimum (dotted line) temperatures from 1 January to 31 December in 2007 (a) and 2008 (b). Rainfall values are sums, and temperature values are means, over 10-day periods**.**



Differences (in individuals/m^2) between species, zones, sampling dates, development stage, and field zone were determined by fitting a generalized linear model to the data and estimating the dispersion parameter by maximum likelihood using the procedure GENMOD from the SAS software package (SAS Institute 2009) with log as a link function. Species, zone, sampling date, and field zone were considered to be fixed factors. Differences between means were computed, analyzing multiple pairwise differences with Tukey's test. Analyses for differences in insect densities between field zones (interior and edge) based on transect data showed no significance, and consequently, density data from all transects were pooled by field. Subsequent statistical analyses were performed using insect densities per field.

RESULTS AND DISCUSSION

Genera and species of wheat bugs in Sardinia, and geographic distribution

During the two years of sampling in the 13 durum wheat fields of Sardinia, two genera, *Eurygaster* and *Aelia*, and four species, *E. austriaca*, *E. maura*, *A. germari*, and *A. acuminata*, were identified. Other species of *Eurygaster* and *Aelia* that have been cited in Sardinia, such as *E. hottentotta*, *A. rostrata*, *Aelia notata* (King), and *A. klugii*, were not found. Other species reported in other parts of Italy (Servadei 1952; Tamanini 1988; Faraci and Rizzotti Vlach 1995; Derjanschi and Péricart 2005; Fauna Europaea 2011), such as *E. integriceps*, *Eurygaster testudinaria* (Geoffroy), *Eurygaster dilaticollis* (Dohrn), and *Aelia sibirica* (Reuter), were not found either.

Table 2. Percentage of the different species of the genus *Eurygaster* and *Aelia* collected per year and zone. Values are expressed in percentage of the total number of adults captured during each year (n = 347 in 2007; n = 291 in 2008).

	2007				2008			
	Zone 1	**Zone 2**	**Zone 3**	**Tot.**	**Zone 1**	**Zone 2**	**Zone 3**	**Tot.**
Eurygaster								
E. austriaca	0.6%	54.7%	18.4%	73.7%	1.7%	50.9%	24.1%	76.6%
E. maura	1.4%	7.5%	0.0%	8.9%	0.3%	4.1%	0.3%	4.8%
Tot. *Eurygaster*	***2.0%***	***62.2%***	***18.4%***	***82.6%***	***2.0%***	***55.0%***	***24.4%***	***81.4%***
Aelia								
A. germari	0.9%	9.5%	3.5%	*13.9%*	0.7%	9.3%	3.4%	*13.4%*
A. acuminata	0.0%	3.5%	0.0%	*3.5%*	0.0%	4.5%	0.7%	*5.2%*
Tot. *Aelia*	*0.9%*	*13.0%*	*3.5%*	*17.4%*	*0.7%*	*13.8%*	*4.1%*	*18.6%*
Total Adults	***2.9%***	***75.2%***	***21.9%***	***100%***	***2.7%***	***68.7%***	***28.5%***	***100%***

The results regarding the different species collected from each zone are shown in Table 2, in which data is expressed as percentage of total adults captured per year. The most abundant species was *E. austriaca*, which represented 75.1% of the total number of adults collected, followed by *A. germari* (13.6%), *E. maura* (7.1%), and *A. acuminata* (4.2%). These four species were present in all zones except for *A. acuminata*, which was never found in Zone 1. In all the zones and years, the most abundant species was *E. austriaca*, with an exception in Zone 1 in 2007, where *E. maura* was the most abundant species.

Density of wheat bugs in durum wheat fields

Frequency classes of wheat bug densities

In 2007 and 2008, about 80% and 65% respectively of the fields sampled registered a very low density of wheat bugs, between 0 and 1 individuals/m^2 (Figure 3). In June in both years, the density of wheat bugs was higher compared to May, and approximately half of the fields registered a density that ranged from 0.5 to 2 individuals/m^2 (Figure 3). There were very few fields with densities above 4 individuals/m^2, established as the damage threshold by Paulian and Popov (1980). The exceptions were observed in Zone 2, which had 4.3 and 7.3 individuals/m^2 in field 3 in June 2007 and 2008 respectively, and 8.2 individuals/m^2 in field 8 in June 2008.

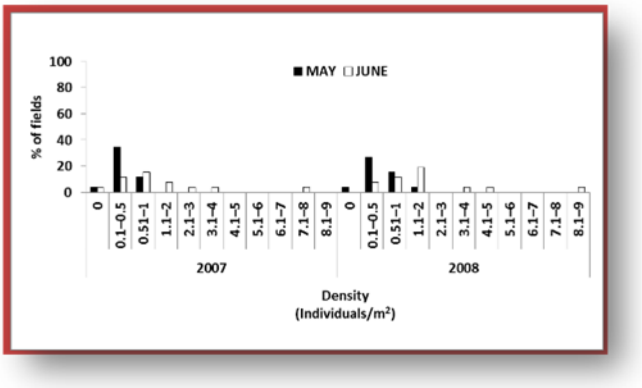


Figure 3. Frequency classes of wheat bug densities (individuals/m^2) found in fields sampled in May and June in 2007 and 2008.

Total density of wheat bugs per year and zone

A total of 867 wheat bugs (638 adults and 229 nymphs) were collected. The average density of wheat bugs sampled in Sardinia in 2007 (0.98 individuals/m^2) was not significantly different (p > 0.05) than 2008 (1.19 individuals/m^2). Population densities, however, varied significantly between zones (Figure 4). The average density in Zone 2 was significantly higher than Zone 3 and Zone 1 (Figure 4). Many factors influence the optimal conditions for the development of wheat bugs, such as climatic conditions, areas cropped, parasites, and overwintering sites (Javahery 1996; Popov et al. 2003; Kutuk et al. 2010), and they can determine different population densities. During the period 20 April – 20 June, immediately before the first sampling (1–10 May) until the second sampling (10–20 June), the average temperature in Zone 2 was the highest (in 2007: Zone 2, 19.3º C; Zone 3, 18.7º C; Zone 1, 18.5º C; in 2008: Zone 2, 18.7º C; Zone 3, 18º C; Zone 1, 17.5º C). In both years, during the period considered above, the average maximum temperature was highest in Zone 2, whereas Zone 1 always presented the lowest average minimum temperature. Higher temperatures stimulate the development of wheat bugs (Javahery 1996; Iranipour et al. 2010) and could explain the different densities in the three zones (Figure 4).

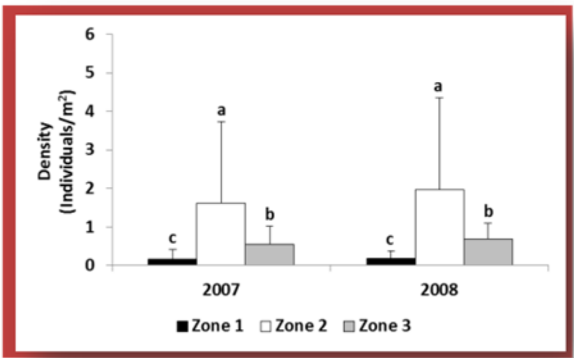


Figure 4. Mean densities of wheat bugs per year in the different zones. In each year, the same letter indicates no significant differences (p < 0.05). Bars represent standard deviation (n = 312).

The most abundant genus, statistically significant in both years of the study, was *Eurygaster*, with 0.80 individuals/m^2 in 2007 and 0.83 individuals/m^2 in

2008, while *Aelia* had a density of 0.18 individuals/m^2 and 0.37 individuals/m^2 in 2007 and 2008 respectively ($p < 0.05$).

The population density of each genus (Figure 5) showed similar results as total density. In both years, the density of the genus *Eurygaster* was significantly higher in Zone 2 and Zone 3 compared to Zone 1. Similarly, the density of the genus *Aelia* was always significantly higher in Zone 2 than in Zone 3 and Zone 1. The density of *Aelia* was higher in Zone 3 than in Zone 1 in 2007 and 2008 (Figure 5).

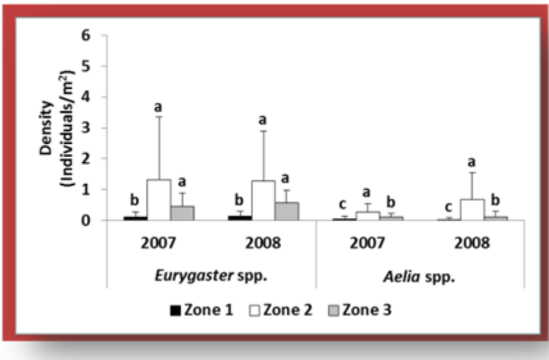


Figure 5. Mean densities of *Eurygaster* spp. and *Aelia* spp. per year and zone (B). Within each genus and year, the same letter indicates no significant differences ($p < 0.05$). Bars represent standard deviation (n = 312).

Density of wheat bugs by sampling date (May–June) and by development stage (nymphs and adults)

Densities of wheat bugs were statistically lower ($p < 0.05$) in the first sampling date (May) than in the second sampling date (June) in Zone 1 (in 2007, 0.07 individuals/m^2 and 0.26 individuals/m^2 respectively; in 2008, 0.03 individuals/m^2 and 0.33 individuals/m^2 respectively) and Zone 2 (in 2007, 0.38 individuals/m^2 and 2.84 individuals/m^2 respectively; in 2008, 0.49 individuals/m^2 and 3.45 individuals/m^2 respectively). According to the wheat bugs' life-cycle, they overwinter in or under diverse shelters (stones, dry leaves, grass clumps) until they move to cereal fields to feed and mate. Few adults were found in wheat fields in May (< 1 individuals/m^2). No nymphs were found in May, as reproductive activity had not yet begun. It is important to know the density of the overwintered adults in wheat fields because it is associated to nymphs and new-adults generation (Kutuk et al. 2010), which is the most detrimental to wheat crop.

In June, when wheat was at the end of grain-filling and maturation, overwintered adults had already reproduced and a mixed population of nymphs and adults was found, resulting in a higher population density with respect to May, as explained above. These findings, regarding wheat bugs densities in May and June, agree with other studies, such as Hariri et al. (2000) in Syria, Popov et al. (2003) in Romania, and Kutuk et al. (2010) and Canhilal et al. (2005) in Turkey.

The genus *Eurygaster* showed significantly higher densities ($p < 0.05$) in June than in May only in Zone 2 in 2007 and in all the zones in 2008 (Figure 6A). The densities of the genus *Aelia* were significantly higher in Zone 2 in June of both years (Figure 6B).

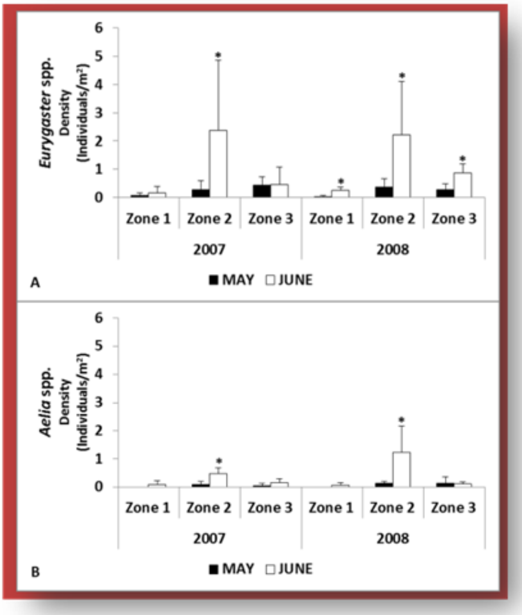


Figure 6. Mean density of *Eurygaster* spp. (A) and *Aelia* spp. (B) per year in the different zones in May and June. Data followed by an asterisk (*) indicates significant differences ($p < 0.05$) within each zone in each year. Bars represent standard deviation (n = 312 for each genus).

As regards the density of adults and nymphs in June, significantly more *Eurygaster* spp. adults than nymphs were recorded in Zone 2 in 2007 and in Zone 3 both years (Figure 7A). For *Aelia* spp., significantly more adults than nymphs were found in Zone 2 and Zone 3 in 2007 (Figure 7-B). The only exception is for *Aelia* spp. in Zone 2 in 2008, when significantly more nymphs than adults were found (Figure 7B). Finally, in some cases no nymphs were recorded from the genus *Eurygaster* in Zone 1 in 2007 and from the genus *Aelia* in Zone 1 for both years (Figure 7AB).

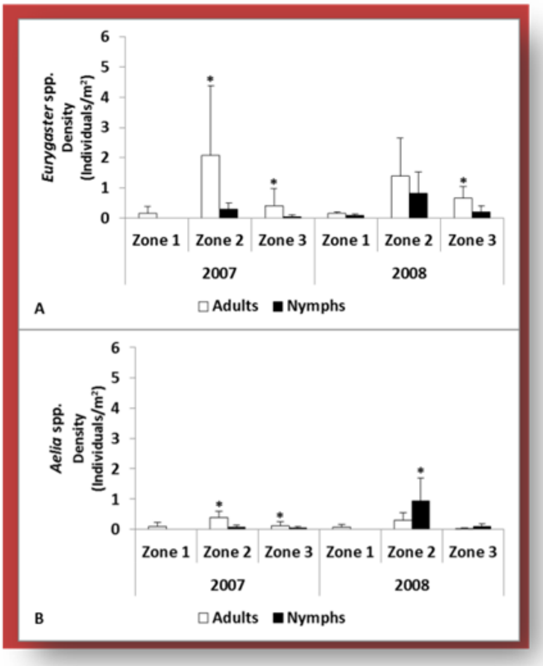


Figure 7. Mean densities of adults and nymphs of *Eurygaster* spp. (A) and *Aelia* spp. (B) in June. Data followed by an asterisk (*) indicates significant differences (p < 0.05) within each zone in each year. Bars represent standard deviation (n = 312 for each genus).

Density by field zone: edge or interior

No significant differences in density between the edge and the interior of the field were found for either *Eurygaster* spp. or *Aelia* spp., neither in any of the years studied nor in the two sampling periods, except for *Aelia* spp. in June 2007 in Zone 2, in which its density was significantly higher (p < 0.05) in the edge of the field (0.63 individuals/m^2) than in the interior (0.31 individuals/m^2). This result was possibly due to the fact that many sampled fields were side by side in a continuum without a properly limiting edge. Conversely, in other studies (Afonina et al. 2001; Pérez-Rodriguez et al. 2008) in which the wheat fields were sampled in the edge and in the interior, differences were found between the field zones. In a region of South Russia, for example, *E. integriceps* began to colonize the field from its edges; however, the following generations were more abundant in the center of the field (Afonina et al. 2001). In northeast Spain, differences between density in the edge and the interior of the field were found only for *Aelia* spp., but not for *Eurygaster* spp. (Pérez-Rodriguez et al. 2008). It is important to know the distribution of the wheat bugs in fields in order to select the appropriate sampling method to be used. This could, moreover, permit the early detection of the wheat bugs before copulation and oviposition, which is very important for the control of this pest.

CONCLUSIONS

The predominant species of wheat bug found in the durum wheat fields sampled in Sardinia was *E. austriaca*, followed by *A. germari*, *E. maura*, and *A. acuminata*. Bug density varied significantly according to the zone, being much higher in Zone 2 than in Zone 1 and Zone 3. The average density of bugs was low (1.1 individuals/m^2), but in certain areas it was above the damage threshold (4 individuals/m^2). Therefore, it would be necessary to monitor the wheat bugs in order to detect outbreaks before they produce economic damage and spread to other areas. The overall density of wheat bugs was lower in May than in June. No nymphs were found in May, only adults. No significant differences were found in the distribution of bugs between field edge and interior, except for *Aelia* spp. in Zone 2 in 2007.

Considering how important durum wheat crops are to the Italian economy, and in view of the infestations of wheat bugs in several Italian regions, it would be desirable to carry out more studies on wheat bugs in the durum wheat production areas. A better understanding of the spatial-temporal population trends is needed in order to develop and apply a cost-effective and environmentally sound pest management program for the control of these pests.

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CAPÍTULO 2

Alteración de las prolaminas en trigo duro por especies del género *Eurygaster* y *Aelia* (Insecta, Hemiptera)

Resumen

Las chinches de los cereales tienen una amplia distribución en diversas áreas de Europa, Asia y Norte de África. Especies pertenecientes a los géneros *Eurygaster* y *Aelia* se alimentan picando los granos de trigo afectando a la calidad de las proteínas. Esta alteración se ha estudiado principalmente en trigo blando (*Triticum aestivum* L.). El presente estudio aporta información sobre la degradación de las prolaminas (gluteninas y gliadinas) del trigo duro (*Triticum turgidum* L. var *durum*) dañado por los chinches de los cereales en seis variedades cultivadas en Cerdeña (Italia). Muestras de harina integral, con un 70% de trigo sano y un 30% de trigo dañado se incubaron a dos temperaturas (45 y 4°C), y a diferentes tiempos (0, 1 y 3 h). El contenido de las gluteninas y gliadinas se analizó mediante electroforesis capilar zonal. La presencia de granos picados por los chinches de los cereales influyó en la calidad de las proteínas del trigo duro. Las gluteninas se degradaron rápidamente, con independencia de la temperatura de incubación. En cambio, la degradación de las gliadinas resultó ser dependiente de la temperatura y del tiempo. Ante estos resultados, cabe plantearse la posibilidad de que la degradación de las gluteninas no sea debida sólo a la actividad de las proteasas sino también a algún otro factor ligado a la actividad alimenticia de los chinches de los cereales, todavía por determinar.

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Prolamin proteins alteration in durum wheat by species of the genus *Eurygaster* and *Aelia* (Insecta, Hemiptera)

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ABSTRACT

Wheat bugs are widely distributed in various areas of Europe, Asia and North Africa. Species belonging to the genus *Eurygaster* and *Aelia* pierce wheat kernels affecting protein quality. The effects of these insects' feeding activity have been studied mainly in bread wheat (*Triticum aestivum* L.). This study provides information on the degradation of prolamin proteins (glutenins and gliadins) of bug-damaged durum wheat (*Triticum turgidum* L. var *durum*) in six cultivars grown in Sardinia (Italy). Samples of whole flour mixture of 70% sound wheat and 30% damaged wheat were hydrated and incubated at two temperatures (45 and 4°C), for different periods of time (0, 1 and 3 h). Glutenin and gliadin content was analysed using free zone capillary electrophoresis. The presence of bug-damaged kernels had influence on the quality of durum wheat proteins. Glutenins were rapidly degraded independently to incubation temperature. Gliadins degradation, however, took place with dependence on temperature and incubation time. Therefore glutenin degradation was possibly not due solely to the activity of proteolytic enzymes

but also to some other as yet unknown factor linked to wheat bugs' feeding activity.

Abbreviations used: A-PAGE (acid polyacrylamide gel electrophoresis), DTT (dithiothreitol), FZCE (free zone capillary electrophoresis), HMW-GS (high molecular weight glutenin subunits), IDA (iminodiacetic acid), LMW-GS (low molecular weight glutenin subunits), RP-HPLC (reverse phase – high performance liquid chromatography)

INTRODUCTION

Wheat (*Triticum* spp.) is one of the three most important crops in relation to cultivated area (approximately 215 million hectares) and total global production (approximately 600 million tonnes) (FAOSTAT, 2008). The unique properties of wheat flour and dough enable the production of a wide range of products such as various types of bread, pasta, cakes and biscuits (Shewry *et al*., 1997).

Durum wheat (*Triticum turgidum* L. var *durum*) is a very important crop in Italy, the production in 2007 was approximately 4.1 million tonnes (ISTAT, 2008), around 15% of the world production. Durum wheat is used mainly for the manufacture of pasta as well as for baking traditional types of bread (Quaglia, 1988). The semolina obtained from durum wheat is the preferred prime matter for the manufacture of superior quality pasta (Feillet and Dexter, 1996).

Different factors can be detrimental to wheat crops, agronomical factors, environmental conditions, pests, diseases, etc., which can result in loss of quantity and quality of wheat, semolina or flour. Wheat bugs of the genus *Eurygaster* (Hemiptera, Heteroptera, Fam. Scutelleridae) and *Aelia* (Hemiptera, Heteroptera, Fam. Pentatomidae), also known as Sunn pest, affect both aforementioned aspects.

Eurygaster and *Aelia* wheat bugs are widely distributed in various areas of Europe, Asia and North Africa (Paulian and Popov, 1980) and an estimate of more than 15 million hectares of cereals (mainly wheat and barley) are infested annually in Syria, Iraq, Iran, Turkey, Afghanistan and Lebanon, as well as in Central Asia and the Caucasus, Bulgaria and Romania (El Bouhssini *et al*., 2002). There are long term studies focussed on integrated pest control (El Bouhssini *et al*., 2002; Parker *et al*., 2003) and on the effects on protein alteration caused by the salivary residues left in the kernels by the insects' feeding (Kretovich, 1944; Karababa and Ozan, 1998; Sivri *et al*., 1998, 1999, 2004; Hariri *et al*., 2000; Aja *et al*., 2004; Caballero, 2005; Ozderen *et al*., 2008; Werteker and Kramreither, 2008). Effects on the alteration of starch granules have been reported for *Nysius* spp. (Hemiptera, Heteroptera, Fam. Lygaeidae), a wheat bug present in Australia and New Zealand, (Every *et al*., 1990; Lorenz and Meredith, 1998) but amylase activity is not involved in the wheat damage caused by *Aelia* spp. and *Eurygaster* spp. (Rosell *et al*., 2002a).

Both nymph and adult *Eurygaster* and *Aelia* wheat bugs insert their piercing-sucking mouthparts in the wheat kernels and extract the substances within. In order to facilitate the suction of the nutritional elements of the endosperm, the kernels are digested externally by injecting saliva rich in proteolytic enzymes (Sivri *et al*., 1998) and amylases (Kazzazi *et al*., 2005). Pierced kernels in the field usually continue to mature. When ripe, bug-damaged kernels present a whitish opaque spot and sometimes also a small black dot where the kernel was pierced (Hariri *et al*., 2000).

There is a general agreement that durum wheat protein content is the primary factor influencing rheological properties and pasta quality (D'Egidio *et al*., 1990; Novaro *et al*., 1993; Feillet and Dexter, 1996). Protein content and amino acid composition of wheat vary depending mainly on genotype and agro-climatic conditions (Lopez-Bellido *et al*., 1998; Rharrabti *et al*., 2003; Dupont *et al*., 2006).

Gluten proteins are to a considerable extent responsible for the functional properties of flour. Wheat gluten consists of more than 50 protein components (Shewry *et al*., 1987) that have been traditionally classified into two groups, gliadins and glutenins (Wieser, 2007). Gliadins are soluble in aqueous alcohols (60-70% ethanol, 50% 1-propanol) and are present as monomeric proteins that lack inter-chain disulphide bonds, presenting instead intra-chain disulphide bonds (Shewry *et al*., 1997). Glutenins are insoluble in aqueous alcohols and consist of protein subunits present in polymers stabilised by inter-chain disulphide bonds. A reduction of these bonds results in subunits which are soluble in alcohol/water mixtures. Rheological properties of dough depend on both protein fractions (Shewry *et al*., 1997).

Although the effects of wheat bugs' feeding activity on bread wheat have been largely studied (Karababa and Ozan, 1998; Sivri *et al*., 1999, 2004; Hariri *et al*., 2000; Aja *et al*., 2004; Vaccino *et al*., 2006; Werteker and Kramreither, 2008) there are not many works regarding durum wheat and other species of the genus *Triticum*. There are various studies on the percentage of bug-damaged kernels necessary in order to seriously affect the kernel, flour, dough or bread quality parameters. Wheat samples which had more than 5% bug-damaged kernels changed their physicochemical properties and showed significantly lower quality (Karababa and Ozan, 1998; Hariri *et al*., 2000). The aim of this study was to investigate how the feeding activity of cereal bugs of the genus *Eurygaster* and/or *Aelia* affects glutenins and gliadins in durum wheat at different incubation conditions concerning temperature (45 and 4°C) and time (0, 1 and 3 h).

MATERIAL AND METHODS

Wheat samples

Six cultivars (Karalis, Asdrubal, Claudio, Rusticano, Colosseo and Canyon) of durum wheat grown in extensive commercial fields near Villamar in the south-centre of Sardinia island (Italy) were tested. Those samples were provided by Laore, the Regional Agency of Sardinia for the Development of Agriculture. The samples showed a percentage of damaged kernels ranging from 1.6 to 4.1%. In each sample, bug-damaged kernels, characterized by a typical whitish opaque spot and also very often a black dot, were separated visually from sound kernels. Two series of sub-samples were prepared for each cultivar in order to carry out the protein analysis. One of the sub-samples consisted of only sound kernels without visible defects; the other sub-samples consisted of medium level bug-damaged kernels, - *i.e.,* with a damage between 1/3 and 2/3 of the total grain surface-, lacking other visible defects. The sound kernels were ground using a Perten 3100 laboratory grinder to obtain wholemeal flour, whereas a Culloti laboratory grinder was used for the damaged kernels because of their scarcity. Both mills had a 0.8 mm sieve.

Protein alteration test, capillary electrophoresis analysis

For each cultivar, two types of samples of whole flour were worked out: on one hand, samples of sound flour (named "sound wheat", used to perform a control or stability test), and on the other hand samples of a blend of 70% sound flour and 30% bug-damaged flour (named "damaged wheat" used to perform a degradation test).

All samples, after hydration with deionised water, were tested using different incubation times (0 h = unincubated, 1 h = 1 hour and 3 h = 3 hours) and temperatures (45 and 4°C). Temperatures tested by authors in studies on bread wheat usually range from 37°C to 45°C (Sivri *et al*., 1998; 1999; Aja *et al*., 2004), whereas 4°C is a temperature that has not been tested previously.

The proportions of the blend of wheat , 70% sound wheat and 30% damaged wheat, as well as incubation conditions at 45°C were selected in order to provide a sufficient degree of protein degradation and also because they had already been tested on bread wheat (Sivri *et al*., 1999) therefore providing a comparative frame to our results. Incubation at 4°C was chosen to assess if degradation can be blocked. All the assays were carried out at least in duplicate.

A sequential protein extraction (albumins, globulins, gliadins and glutenins) was carried out on each sample following the protocol described by Bean and Lookhart (1998). For each cultivar, a sample of 100 mg of sound wheat or damaged wheat was hydrated with 1 mL of deionised water for incubation or to start off protein fractioning, specifically albumins. After stirring for 5 min the sample was centrifuged at 14000 rpm for 5 min; the supernatant was discarded and the pellet resuspended using 1 mL of extraction buffer (50 mM Tris HCl, 50 mM KCl, 5 mM EDTA, at pH 7.8) so as to discard globulins; this operation was repeated twice, always stirring for 5 min and centrifuging at 14000 rpm. The pellet was then resuspended using 1 mL of 50% 1-propanol (v/v) in order to extract the gliadins. The pellet was cleaned yet again with 1 mL of 50% 1-propanol. Finally, the glutenins were obtained by resuspending and stirring the pellet with 50% 1-propanol + 1% DTT (v/v) for 30 min and then centrifuging at 14000 rpm for 5 min. For the samples incubated at 4°C, this temperature was maintained up to albumin and globulin extraction to avoid possible activity of the proteases on the gliadin and glutenin fraction. Gliadin and glutenin extracts were filtered at 45 µm.

Analysis of the different gliadin and glutenin subunits was carried out using FZCE. The process followed was similar to the method described by Bean and Lookhart (2000), but adjusted to a Hewlett-Packard CE and using a silex capillary tube (34 cm Polymicro Phoenix AZ, 25.5 cm $L_{D,}$ 50 µm i.d.) 50 mba × 8 s for injection of the samples, an IDA buffer (50 mM IDA, 20% acetonitrile and 0.05% hydroxypropylmethylcellulose) at 30 kV and 45°C.

Gliadin and glutenin alteration was assessed by comparing the electropherograms obtained from the sound wheat and damaged wheat in the incubation conditions described. The alterations in the total area (total quantity of gliadins or glutenins expressed in mAU*s) of the electropherograms were considered to be signs of protein alteration.

Statistical analysis

The results express the mean values obtained from two repeated trials. Factorial analysis of variance of glutenins and gliadins was carried out using the factors cultivar, temperature and incubation time. The results were analyzed using the Tukey method. The statistical analysis of data was carried out using the SAS software. A level of significance of 0.05 ($P<0.05$) was used throughout the study.

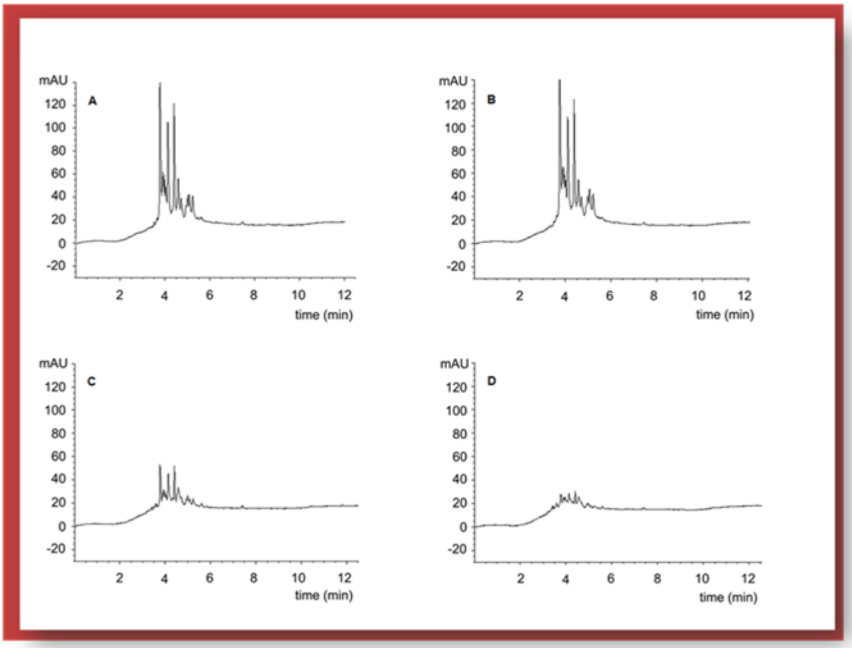

Figure 1. FZCE electropherograms of cv. Karalis glutenins (50% 1-propanol + 1% DTT) from sound wheat unincubated (A) and incubated at 45ºC 3 h (B), and damaged wheat incubated at 45ºC for 1 h (C) and 3 h (D).

RESULTS

Glutenins

The stability test proved that glutenins from sound wheat were stable, with no significant differences in the amount of glutenins between the sound wheat unincubated and the sound wheat incubated for 3 h at 45°C. This allowed to use the mean value of duplicated sample of unincubated sound wheat as a reference value in order to evaluate degradation; an example of this can be observed in the stability test of cv. Karalis, shown by the equivalent electropherogram area in Fig. 1A-1B. The degradation test proved (Fig. 1C-1D) that the glutenins of the damaged wheat, on the other hand, were degraded rapidly even in unincubated samples (Table 1, Fig. 2), with an average loss compared to the sound wheat samples of -65%. These differences were significant in all wheat cultivars when compared to the sound wheat (Fig. 2).

Table 1. Quantification of the alteration of total glutenins and gliadins of the damaged wheat at different temperatures (45°C, 4°C) and different incubation times (0 h, 1 h and 3 h) compared to sound wheat, expressed as loss (-) or gain (+) of area, in percentage and mAU*s (total area). For each cultivar, data within columns followed by the same letter are not significantly different (*P*<0.05).

	Karalis		Asdrubal		Claudio		Rusticano		Colosseo		Canyon		Mean*
	%	mAU*s	%	mAU*s	%	mAU*s	%	mAU*s	%	mAU*s	%	mAU*s	%
Total Glutenins													
Sound wheat		3718 a		4250 a		3822 a		3950 a		3823 a		4418 a	
Damaged wheat 0 h 4°C	-63	-2345 b	-60	-2554 b	-65	-2472 b	-58	-2342 b	-73	-2795 b	-69	-3047 b	-65±5.5
Damaged wheat 1 h 45°C	-75	-2779 c	-73	-3084 c	-82	-3143 c	-66	-2611 c	-84	-3198 c	-87	-3853 cd	-78±8.0
Damaged wheat 1 h 4°C	-79	-2920 cd	-70	-2992 c	-85	-3234 cd	-74	-2926 d	-84	-3224 c	-84	-3704 d	-79±6.0
Damaged wheat 3 h 45°C	-80	-2987 cd	-86	-3641 d	-88	-3346 cd	-80	-3157 de	-91	-3489 d	-91	-4009 c	-86±4.9
Damaged wheat 3 h 4°C	-82	-3053 d	-81	-3454 e	-90	-3451 d	-83	-3271 e	-91	-3492 d	-91	-4008 c	-86±4.8
Total Gliadins													
Sound wheat		2958 ab		3511 a		3179 a		3242 ab		3149 ac		3862 a	
Damaged wheat 0 h 4°C	+4	+114 a	-11	-386 ac	+5	+175 a	+5	+167 a	+30	+943 b	-9	-330 a	+4±14.6
Damaged wheat 1 h 45°C	+11	+334 a	-18	-637 abc	-32	-1023 b	-22	-718 b	-18	-559 a	-38	-1485 b	-20±17.2
Damaged wheat 1 h 4°C	-9	-264 ab	-16	-557 abc	-6	-179 a	+8	+250 a	+17	+551 bc	-18	-689 ac	-4±13.8
Damaged wheat 3 h 45°C	-56	-1652 c	-41	-1438 b	-55	-1763 c	-50	-1624 c	-70	-2193 d	-66	-2563 d	-56±10.5
Damaged wheat 3 h 4°C	-24	-718 b	-31	-1098 c	-6	-205 a	-5	-160 ab	-11	-332 a	-35	-1342 bc	-19±13.1

* Mean: mean of the alteration of the six cultivars ± standard deviation.

Degradation was slightly higher when the damaged wheat samples were incubated (Table 1). The average degradation for 1 h was -79% at 4°C and -78% at 45°C. The average degradation for 3 h at both 4°C and at 45°C was -86% (Table 1). Differences between sound wheat samples and incubated (1 h and 3 h) damaged wheat samples were significant for all cultivars (Fig. 2). Not all samples showed significant differences between incubation at 1 h or 3 h at the same temperature (Fig. 2). For each cultivar, there were no significant differences between incubation at 45°C and at 4°C for the same incubation time, with the exception of Rusticano and Asdrubal cultivars incubated for 1 h and 3 h respectively (Table 1). Thus it can be stated, with some exceptions, that glutenins were degraded to the same degree at 45°C as at 4°C.

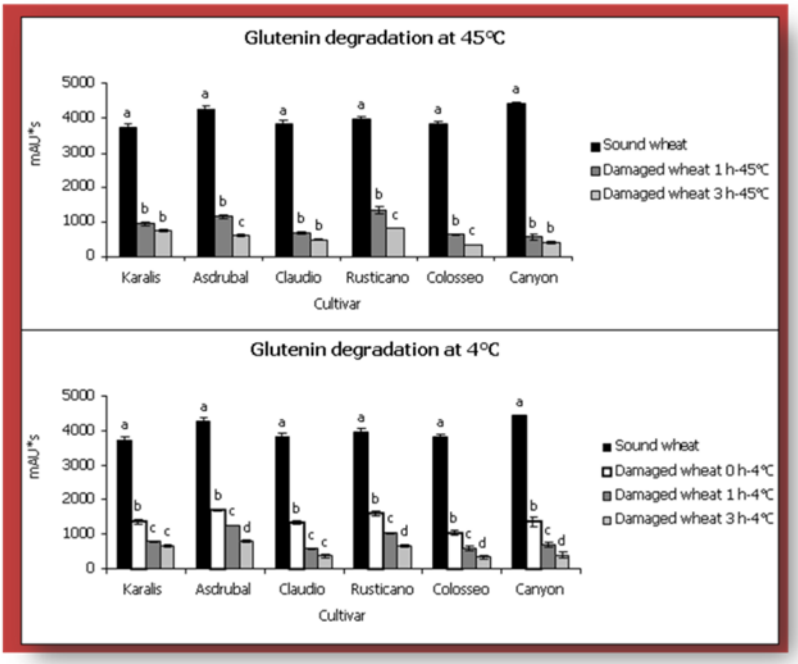

Figure 2. Results for glutenin degradation, comparing sound wheat and damaged wheat, in six durum wheat cultivars, at different temperatures (45 and 4°C) and different incubation times (0 h, 1 h and 3 h) expressed as total area in mAU*s. Bars describe standard deviation. For each cultivar at 45°C or at 4°C, the same letter indicates not significant differences (Tukey test, *P*<0.05).

Gliadins

The stability test proved that gliadins from sound wheat were stable, with no significant differences in the amount of gliadins between the sound wheat unincubated and the sound wheat incubated for 3 h at 45°C. This allowed to use the mean value of the duplicated sample of unincubated sound wheat as a reference value in order to evaluate degradation; an example of this can be observed in the stability test of cv. Karalis shown by the equivalent electropherogram area in Fig. 3A and 3B. The behaviour of gliadins in the damaged wheat samples differed strongly from that of glutenins. It was found that, in contrast to that of glutenins, degradation of gliadins was much lower and was time and temperature dependent (Table 1, Figs. 3C-3D and 4). Most of the unincubated (0 h) damaged wheat samples showed a total gliadin area increase (Table 1, Fig. 4), presumably due to the depolymerised glutenins. These increments ranged from +4% to +30% compared to the sound flour, with the exception of the cvs. Asdrubal and Canyon that lost -11% and -9%, respectively (Table 1).

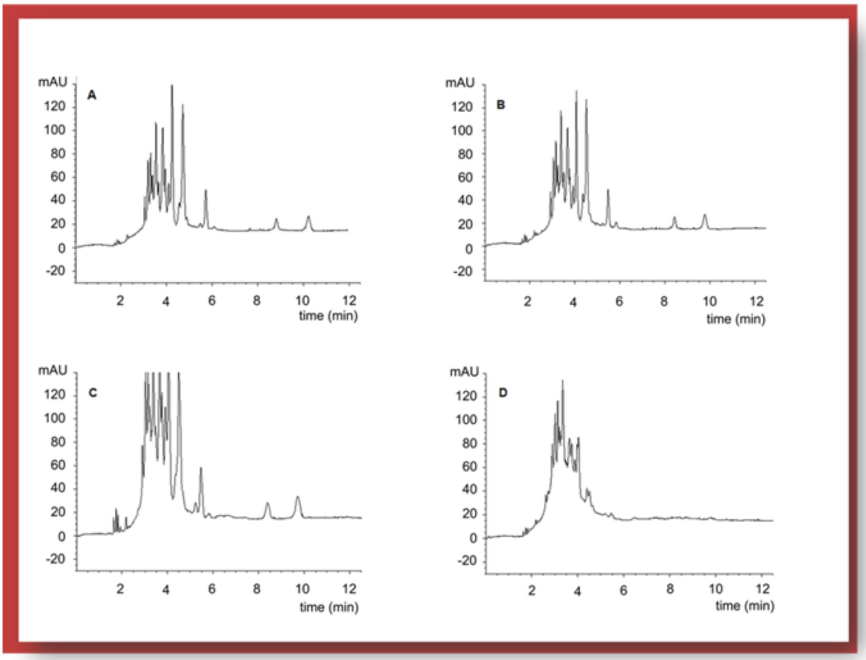

Figure 3. Z FZCE electropherograms of cv. Karalis gliadin (50% 1-propanol + 1% DTT) from sound wheat unincubated (A) and incubated at 45ºC 3 h (B), and damaged wheat incubated at 45ºC for 1 h (C) and 3 h (D).

These differences, however, compared to the sound wheat, were only significant for the Colosseo cultivar (Fig. 4). For the damaged wheat samples incubated for 1 h at 45°C degradation ranged from -18% to -38% with the exception of the cv. Karalis where an increase of +11% was registered (Table 1). The differences were only significant, compared to the sound wheat, in the cvs. Claudio and Canyon (Fig. 4). There were minor variations in the damaged wheat samples incubated for 1 h at 4°C (from +17% to -18%, Table 1) and no significant differences when compared to the sound wheat (Fig. 4). However increases in gliadin degradation were significant for all cultivars, compared to the sound wheat, when damaged wheat samples were incubated for 3 h at 45°C (Fig. 4) In this case the average degradation percentage was -56% of the total gliadins (Table 1). Degradation of gliadins incubated for 3 h at 4°C, with an average degradation of -19%, was much lower than in samples incubated for 3 h at 45°C (Table 1). For each cultivar, there were significant differences between incubation at 45°C and at 4°C for 3 h. Above all, it can be stated that gliadins were not degraded to the same degree at 45°C as at 4°C.

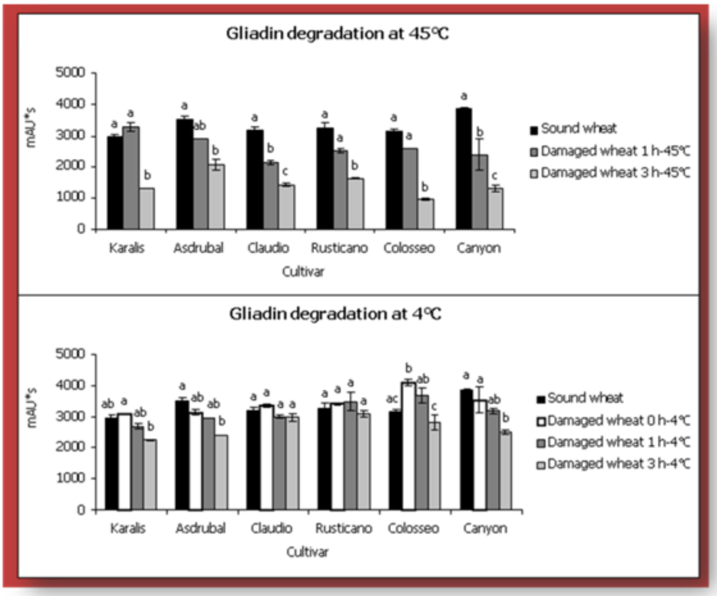

Figure 2. Results for gliadin degradation, comparing sound wheat and damaged wheat, in six durum wheat cultivars, at different temperatures (45 and 4°C) and different incubation times (0 h, 1 h and 3 h) expressed as total area in mAU*s. Bars describe standard deviation. For each cultivar at 45°C or at 4°C, the same letter indicates not significant differences (Tukey test, P<0.05).

DISCUSSION

The previous results show that wheat bugs have a strong influence on durum wheat proteins determining quality loss. Total glutenin and gliadin alteration, due to the presence of bug-damaged kernels, was quantified and results were similar to those described in bread wheat (analysis perfomed only at high temperature). Glutenin results were very much alike to those obtained in bread wheat (Sivri *et al*., 1998, 1999; Rosell *et al*., 2002b). In wheat cultivated in Turkey and manually infested by *E. maura* (L.) bugs, it was seen that after 30 min incubation, more than 80% glutenin was degraded, and increase of degradation was very low 1 h and 2 h after incubation (Sivri *et al*., 1999). In that research, the proportions (2:1) of sound and damaged wheat in the blend were similar to those used in the present study, temperature of incubation was 37°C, and determination of reduced glutenins was done by (RP-HPLC). In bug-damaged bread wheat cultivated in Spain, a decrease of the glutenin fractions HMW-GS and LMW-GS determined with FZCE was also reported (Rosell *et al*., 2002b). Regarding bug-damaged wheat, less information is available on gliadins than on glutenins. The initial increase in the area of gliadins observed in this study, probably due to the presence of glutenin degradation products with a higher electrophoretic mobility, was also described in bug-damaged bread wheat, as in the case of Spanish bread wheat samples and using FZCE (Rosell *et al*., 2002b). In gliadins from bread wheat from Turkey incubated at 37°C, and analysed by A-PAGE, a decrease was also stated, concerning some new bands as well as the original gliadin bands; as in the case of durum wheat, the changes were more obvious with increasing incubation times and most of the gliadin bands were lost after 120 or 240 min of incubation (Sivri *et al*., 1998).

In bug-damaged bread wheat a marked increase of free thiol groups during initial incubation was described (Perez *et al*., 2005). Results in durum wheat damaged by wheat bugs indicate that the alteration of the gliadins and glutenins initially begins with a fast depolymerisation of glutenins, independently of temperature, presumably due to a reduction of inter-chain disulphide bonds. The alteration continues, depending on the temperature, showing a general and specific degradation of gliadins and solubilised glutenins. Therefore, degradation of glutenins was possibly due not only to the action of protease enzymes, but also to another not yet determined salivary agent(s), that would act as a reducer. These durum wheat results could explain the modification of pasta (spaghetti) quality characteristics, referring to a loss of gluten quality, cooking values and a deterioration of sensory properties, when semolina from bug-damaged wheat is used to make pasta (Ozderen *et al*., 2008; Köksel *et al*., 2009). Similar consequences could be expected when baking certain traditional breads with flour obtained from damaged durum wheat, although no test has yet been performed to assess this hypothesis.

The final conclusion is that wheat bugs have a strong influence on durum wheat proteins that determines a quality loss. When hydrating durum wheat flour or semolina obtained from kernels damaged by wheat bugs the glutenins depolymerised quickly and almost in their totality. This degradation is independent of temperature. By contrast, the gliadins and the solubilised glutenins are degraded with less intensity and the degradation is time and temperature dependent.

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CAPÍTULO 3

Efectos de las chinches de los cereales sobre el trigo duro (*T. turgidum* L. var. *durum*): desde el grano hasta la pasta

Resumen

El trigo duro (*Triticum turgidum* L. var *durum*) es uno de los cereales más importantes y es la materia prima preferida para la fabricación de pasta de calidad superior. El trigo duro no está libre de los ataques de los chinches del trigo (Hemiptera, Heteroptera), una plaga con especies de los géneros *Eurygaster* (Fam. Scutelleridae) y *Aelia* (Fam. Pentatomidae), ampliamente distribuida en varias zonas de Europa, Asia y el Norte de África. El objetivo de este estudio fue evaluar los efectos de las chinches de los cereales sobre la calidad del grano de trigo duro, de la sémola y de los parámetros de cocción de la pasta. Dos cultivares, Iride y Simeto, se pusieron a prueba, comparando muestras sanas y tres porcentajes diferentes de granos dañados por las chinches de los cereales (2,5%, 5% y 10%), unos porcentajes que se han encontrado con bastante frecuencia en trigos cosechados. Los análisis de calidad más comunes en el trigo duro se llevaron a cabo en granos, harina integral, sémola y pasta. Se encontró una reducción significativa en la calidad de los granos por la disminución del peso hectolítrico, del peso de mil granos y del test de sedimentación-SDS. La calidad de la sémola disminuyó sobre todo teniendo en cuenta los parámetros alveográficos W y P, en gran medida reducido ya al 2,5% de granos dañados por las chinches de los cereales. El índice de Gluten (GI) y el tiempo de estiramiento se redujeron a partir del 5% de granos dañados por las chinches de los cereales. Los parámetros del Mixolab vinculados con la calidad de la proteína de la sémola (C2, C1-C2) bajan, lo que indica un debilitamiento de la proteína a partir del 5% de granos dañados por las chinches de los cereales. A pesar del claro efecto las chinches de los cereales en la calidad de la sémola, la calidad de los "spaghetti" no se redujo ni siquiera con un 10% de granos dañados por las chinches de los cereales, teniendo en cuenta la absorción de agua, la pérdida en cocción, la materia orgánica total (TOM) y los parámetros de evaluación sensorial. Serían útiles otras investigaciones sobre los efectos de las chinches de los cereales en la

calidad de los "spaghetti", teniendo en cuenta el mismo porcentaje de granos dañados, pero probando una gama más amplia de cultivares de trigo duro y considerando el uso del tiempo de cocción óptimo. Se sugieren también estudios sobre los efectos de las chinches de los cereales en la panificación con sémola, muy común en los países mediterráneos.

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FOOD CHEMISTRY (En Preparación)

Wheat bugs effects on durum wheat (*T. turgidum* var. *durum*): from grain to pasta

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ABSTRACT

Durum wheat (*Triticum turgidum* L. var *durum*) is one of the most important cereal crops and it is the preferred raw material for the manufacture of superior quality pasta. Durum wheat is not free of the attacks of pests, among which wheat bugs (Hemiptera, Heteroptera), a pest belonging to the genus *Eurygaster* Fam. (Scutelleridae) and *Aelia* (Fam.Pentatomidae), widely distributed in various areas of Europe, Asia and North Africa. The aim of this study was to evaluate the effects of wheat bugs damage on durum wheat grains and derived products (semolina and pasta). Two cultivars Iride and Simeto, were tested, comparing sound sample and three different levels of wheat bugs damaged kernels (2.5%, 5% and 10%), found quite commonly in

harvested wheat. The most common quality analyses in durum wheat were carried out on grains, wholemeal, semolina and pasta. A significant reduction in grains quality was found (i.e test weight, thousand kernel weight and SDS test). Semolina quality decreased particularly considering the alveographic parameters W and P, heavily reduced from 2.5% wheat bugs damaged kernels. Gluten index (GI) and stretching time was reduced but from 5% wheat bugs damaged kernels. Mixolab parameters linked with semolina protein quality (C2, C1-C2) lowered, indicating a weakening of protein from 5% wheat bugs damaged kernels. Despite the clear effect of wheat bugs damaged kernel on semolina quality, spaghetti quality was not reduced. Further researches on the effects of wheat bugs in spaghetti quality would be useful, considering the same percentage of wheat bug damaged kernels, but testing a wider array of durum wheat cultivar. Studies about the effects of wheat bugs in bread-making are suggested.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var *durum*) is one of the most important cereal crops. The world production is about 35 million tonnes and Italy is one of the most important producers, generating around 15% of the world production (Taylor and Woo, 2011). Durum wheat is the preferred raw material for the manufacture of superior quality pasta (Feillet and Dexter 1996). The main use of durum wheat is for pasta making, but other traditional foods in the Mediterranean countries are also produced as couscous, bulgur and traditional types of bread (Quaglia 1988). The technological quality of durum wheat therefore depends on semolina yield and on semolina quality, the ability of semolina to be processed into pasta that meets the requirements of the usual consumers (Cubadda 1988). A factor associated with semolina yield is test weight. Test weight is a widely used specification in wheat grading because it is internationally recognized as an index of wheat soundness and an indicator of wheat milling potential (Dexter and D'Egidio 2012). Overall test weight is strongly linked to durum wheat semolina yield for samples of sound physical condition (Watson et al. 1977; Dexter et al. 1987). Thousand kernel weight is another factor often considered to be linked to semolina yield. It is linked with kernel size, density and uniformity although no valid study demonstrates that all varieties of durum wheat with small kernels, and therefore with low thousand kernels weight, have a potentially lower semolina yield (Cubadda 1988). Therefore test weight appears to be a better estimator of durum wheat milling performance than kernel weight (Dexter and Symons 2007).

There is a universal agreement that protein content is the primary factor influencing pasta quality and that gluten strength is an important secondary factor (D'Egidio et al. 1990; Novaro et al. 1993; Feillet and Dexter 1996). Most pasta manufacturers demand a minimum semolina protein content (Feillet and Dexter 1996). Wet and dry gluten content is another frequently used semolina specification. Sodium dodecyl sulphate (SDS)-tests have long been used to characterise wheat quality (Dexter et al. 1980). It is an effective rapid indicator of durum wheat gluten strength (Feillet and Dexter 1996). The gluten strength of semolina can be monitored by different physical dough tests and it has a good relationship with pasta cooking (D'Egidio et al. 1990).

Durum wheat is not free of the attacks of a pest, known as wheat bugs or Sunn pest. Wheat bugs (Hemiptera, Heteroptera), belonging to the genera *Eurygaster* (Fam. Scutelleridae) and *Aelia* (Fam. Pentatomidae) are widely distributed in various areas of Europe, Asia and North Africa (Paulian and Popov 1980). The damage caused by these insects leads to losses of crop and/or yield and quality of wheat. Leaves, stems and ears can be attacked (Critchley 1998). Nymphs and adults of wheat bugs insert their piercing-sucking mouthparts in the kernels and extract the substances within, injecting saliva containing proteases (Sivri et al. 1998; Konarev et al. 2011). If insect pierces the kernel in the early stages of development ("water-ripe", "milk-ripe" stage) when the kernel content is easy to remove, the surface of the kernel appears collapsed around the area of penetration when the grain is mature. These kernels are smaller, lighter and shrivelled, and normally are separated during wheat cleaning processes in the mill and therefore they do not represent a problem in terms of quality (Koksel et al. 2002). If kernel is attacked at a later stage of maturity, the kernel surface is not deformed. The mature damaged kernels are characterized by a whitish opaque spot and a small black dot where the kernel was pierced (Critchley 1998).

It has been shown that the feeding activity of wheat bugs determines wheat flour with reduced bread-making quality (Karababa and Ozan 1998; Hariri et al. 2000; Vaccino et al. 2006). The detrimental effect on baking quality, of injected proteases during wheat bugs feeding process, is very high even in presence of only 3-5% damaged kernels, and dramatically increases for values higher than 10% (Karababa and Ozan 1998; Hariri et al. 2000).

Wheat bugs damage is related with grain and flour yield. The feeding activity of wheat bugs reduces thousand kernels weight, test weight (Karababa and Ozan 1998; Kinaci and Kinaci 2004; Hariri et al. 2000) and also heavily affects the percentage of wheat germination (Bin et al. 2006).

Wheat gluten, as well known, consists of various protein components that have been traditionally classified into two groups, gliadins and glutenins (Wieser 2007). Rheological properties of dough depend on both protein fractions (Shewry et al. 1997).

In bread wheat, wheat bugs infestations affect gluten proteins, gliadins and in particular glutenins (Sivri et al. 1998, 1999; Werteker and Kramreither 2008; Pérez et al. 2005; Rosell et al. 2002a).

There are quite studies, as reported above, related to wheat bugs effects on gluten proteins, physical and rheological properties and baking quality of bread wheat. On the other hand, few studies regarding wheat bugs effects on durum wheat were reported. In particular, it was described the milling properties of durum wheat –with a presence of 20-40% of damaged kernels– by Köksel et al. (2009). It has been also characterized the impact of wheat bugs on durum wheat quality considering the effects on farinograph results (Josephides 1993). Wheat bugs effects were investigated on durum wheat gluten proteins (Salis et al. 2010) and specifically on glutenins (Olanca et al. 2009). The consequences on semolina and pasta quality –with a presence of 20-40% of damaged kernels– were reported by Ozderen et al. (2008) and pasta cooking potential were investigated by Petrova (2000).

The aim of this study is to evaluate the effects of wheat bugs damage on durum wheat grains, semolina and pasta cooking quality, at relative commonly damaged kernels levels (2.5%, 5% and 10%) found in some European countries as Italy (Vaccino et al. 2006; Salis et al. 2010), Spain (Gordún et al. 2008), Austria (Werteker and Kramreither 2008) and Turkey (Kinaci and Kinaci 2004).

MATERIALS AND METHODS

Durum wheat cultivars and sample preparation

Iride and Simeto, two of the most cultivated durum wheat cultivars in Italy in the last years (INRAN 2013), were tested. The wheat samples, 50 kg for each cultivar, were grown in 2007 in the North Sardinia (Italy) and were provided by Agrisardegna S.p.a (Porto Torres, Italy). All wheat samples were cleaned, removing foreign bodies, broken and shrunk kernels. The initial percentage of damaged kernels by wheat bugs in each sample was around 2.5%. The damaged kernels, showing a characteristic mark (a black dot surrounded by a pale or discoloured halo) were separated visually from sound kernels by hand picking by a trained expert. For each cultivar, a sound sample without damaged kernel was prepared and used as a control sample. The damaged and sound kernels were used to obtain, two replicates for each cultivars, of samples damaged at level of 2.5%, 5% and 10%.

Milling durum wheat into semolina and wholemale flour

An amount of 2 kg of the sound (0% damaged kernels) and bug damaged kernels (2.5%, 5% and 10%) samples, for each cultivar, were tempered to 16% moisture content and milled to obtain semolina using a Bühler experimental mill MLU-202 (Uzvill, Switzerland) with three break and three sizing passages. The semolina was purified using a laboratory purifier (Namdad, Italy). An amount of 50 g of grains, for all samples, for each cultivar, was milled using a laboratory grinder Cyclotec mill-PBI (Italy) to obtain wholemeal. Milling was carried out separately for each replicate.

Grain, Wholemeal, Semolina and Pasta Quality Measurements

Different quality analyses were carried out on grains, wholemeal, semolina and pasta. All analyses were performed in duplicate.

Grain

The whole grains samples were evaluated in terms of test weight (TW) and thousand kernels Weight (TKW). TW was determined on the grain samples by the automatic instrument Infratec Grain Analyzer 1241 (FOSS AB Analytical, Sweden) while TKW was determined by counting and weighing 1000 grains.

Wholemeal

The wholemeal samples were characterized by protein content, sodium dodecyl sulfate (SDS) sedimentation test and ash content.

Protein content was determined by the Dumas method (ICC 167, 2000) with the instrument LECO FP528 (USA). SDS-sedimentation test was determined according with the ICC method 151 (1991), with SDS-3%. Ash content was determined according with the ISO method 2171 (1980).

Semolina

Semolina samples were characterized for protein content, wet and dry gluten, gluten index (GI), glutograph test, alveographic indexes - strength or deformation energy (W), tenacity (P), extensibility (L), the ratio P/L (P/L) and swelling index (G); Mixolab parameters (water absorption, stability, C1,C2,C3,C4 and C5) and their differences (C1-C2, C3-C2, C3-C4, C5-C4); yellow index (b*) and brown index (100-L*).

Protein content was determined with the same method as wholemeal. Wet gluten and dry gluten were determined according to ICC 137 (1994). Gluten index was determined according ICC 158 (1995) using Glutomatic System equipment (Perten, Sweden). The stretching time value of gluten was also evaluated with a glutograph (Brabender, Germany) (Sietz, 1987 Alveographic indexes were determined by the NG Chopin Alveograph (Chopin Technologies, France), according to UNI 10453 standard method used for durum wheat semolina, in which dough rested for 18 min.

Semolina was also evaluated by Mixolab, (Chopin Technologies, France). This instrument is able to provide indications about the rheological behavior of protein content and starch gelatinization. The test is performed preparing a constant hydrated dough mass to obtain a target consistency during the first test phase. Dough mixing was carried out at 30°C for 8 min, and then the temperature was increased up to 90°C over 15 min at the rate of 4°C/min. Bowl temperature was held at 90°C for 7 min, cooled to 50°C over 10 min at the rate of 4°C/min, and finally held at 50°C for 5 min. The duration of each assay was 45 min. Figure 1 shows a typical curve recorded in the Mixolab device along the different stages (mixing, heating, and cooling) and the different phases of the curve.

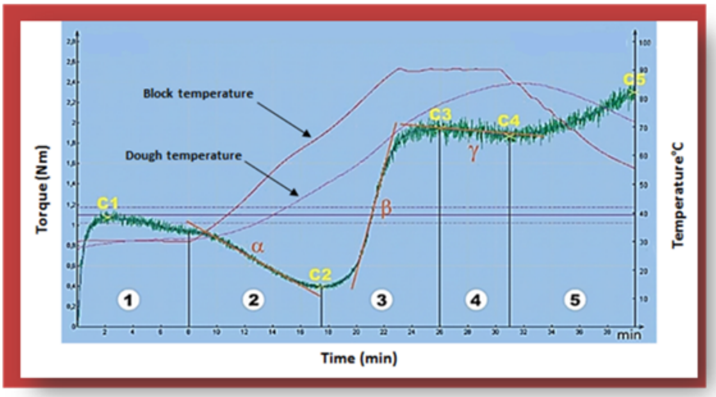

Figure 1. Description of a typical curve obtained in the Mixolab. The numbers indicate the different areas detected in the curve according to the dough changes. Dough development (1-First phase). Protein weakening when temperature increases (2-Second phase). Starch gelatinization (3-Third phase). Amylase activity (4-Fourth phase). Starch gelling due to cooling (5-Fifth phase) (adapted from Chopin Technologies, 2012).

The parameters determined with Mixolab were: Water absorption, amount of water to reach C1=1.1 Nm ± 0.05, during the first phase of the curve; C1 (Nm) is the maximum torque during mixing, in the first phase of the curve and it is used as a calibration; Stability, time during which the upper frame of the curve is higher than C1 less 11% during the first phase of the curve; C2 (Nm) measures the protein weakening based as a function of mechanical work and temperature, in the second phase of the curve; C3 (Nm) measures the starch gelatinization, in the third phase of the curve; C4 (Nm) indicates the hot starch gel stability, in the fourth phase of the curve; C5 (Nm) measures the starch retrogradation in the cooling stage, in the fifth phase of the curve. The differences between the previous parameters provide additional information (Moscaritolo et al. 2008; 2012). C1-C2 is related with gluten strength; C3-C2 is linked with the starting point of starch gelatinization and on the maximum point of starch gelatinization (90 º C); C3-C4 provides guidance on the weakening of the mixture at 90 º C caused by the release of water that is not retained and it is linked with amylase activity; C4-C5 gives information on the dough adhesive capacity.). Semolina color indexes, yellow index (b*) and brown index (100-L*) were measured by a colorimeter Minolta CR 400 (Konica Minolta, Japan).

Pasta

Semolina samples were also used to produce pasta. The semolina was mixed with tap water (total hardness = 18 German degrees) to obtain a total dough water content of 32%. The dough was processed into spaghetti (1.65 mm of diameter, 20 cm of length) using a laboratory press (Namad, Italy) with a capacity of 1.5-3.5 kg. Extrusion condition were temperature of 50±5ºC, pressure of 60±10 atm and vacuum was 700mmHg. A drying cycle of about 20 hours was carried out by an experimental drying system (AFREM-France) employing a low-temperature drying diagram (50°C). Cooking quality was evaluated by water absorption during cooking, cooking loss, total organic matter (TOM), and sensory judgment (SJ).

Cooking test was performed on 100 grams of spaghetti into 1 L of boiling water for a standard time of 13 min. Then, the samples were allowed to drain for 2 min.

Cooking loss, the weight of total solids lost during cooking, was measured by evaporating the cooking water to dryness overnight in a forced-air oven at 110ºC. The residue was weighed and reported as g/l.

Total Organic Matter (TOM) related to the surface material released in the washing water from cooked spaghetti, was determined by a chemical method according to D'Egidio et al. (1982).

Pasta cooking quality was assessed by SJ at a standard cooking time of 13 min which corresponds to an overcooking for 1.65mm diameter spaghetti (Fagnano et al. 2012). The sensory judgment (SJ) was performed by a highly trained panel of three experts. The following textural parameters were considered (D'Egidio and Nardi 1996): firmness, stickiness and bulkiness. Firmness represents the resistance of cooked pasta to chewing by the teeth; stickiness is the material adhering to the surface of cooked pasta evaluated by visual inspection and by handling; bulkiness, which is related to stickiness, is the adhesion degree of pasta strands to each other and it is evaluated visually and manually. Each of these three parameters was evaluated by a score ranging from 10 to 100. For firmness, ≤20=absent, 40=rare, 60=sufficient, 80=good, 100=very good; for stickiness and bulkiness, ≤20= very high, 40=high, 60=rare, 80=almost absent, 100=absent; The score of each sensorial component was the arithmetic mean of three assessors; the final value of SJ was the arithmetic mean of the three textural components: the higher is the SJ value, the higher is the quality. Water absorption during cooking was evaluated nine minutes after draining (D'Egidio et al. 1993).

Statistical analysis

The results express the mean values obtained from two replicates trials. Every analysis was performed at least in duplicate. One-way analysis of variance (ANOVA) for each cultivar firstly and then Tukey's range test were carried out using the factor percentage of damaged kernel (0%, 2.5%, 5% and

10%) for the variables protein content, ash content, SDS-test, TW, TKW, semolina yield, wet gluten, dry gluten, GI, glutopraph stretching time value, yellow index value, brown index value, alveograph values (W, P, L, P/L and G), Mixolab parameters (Water absorption, Stability, C1,C2,C3,C4,C5 and C1-C2, C3-C2, C3-C4 and C5-C4), water absorbed during cooking, cooking loss, TOM, SJ parameters (stickiness, bulkiness, firmness and global value). The statistical analysis of data was carried out using the R software (R Core Team, 2012). A level of significance of 0.05 was used throughout the study.

RESULTS AND DISCUSSION

Physical-chemical quality of grains and yield

The physical-chemical grains characteristics and semolina yield of cultivars Iride and Simeto from sound samples (0% damaged kernels) and different percentage of damaged kernels samples (2.5%, 5% and 10%) are reported in table 1.

Table 1. Wheat bugs damaged kernel effects on protein content, ash, SDS-test, test weight (TW), thousand kernel weight (TKW) and semolina yield are reported. Protein, ash and SDS-test were determined in wholemeal. For each cultivar, data followed by the same letter are not significantly different ($P < 0.05$). Data are mean ± standard deviation.

PHYSICAL-CHEMICAL GRAIN QUALITY CHARACTERISTICS							
CULTIVAR	DAMAGED KERNEL (%)	Protein (%, Dry matter)	Ashes (%, Dry matter)	SDS-test (cm^3)	TW (kg/hL)	TKW (g)	Semolina Yield (%)
IRIDE	0 (Sound)	a12.31 ±0.628	a1.98 ±0.011	a37.00 ±0.000	a84.0 ±0.070	a41.55 ±0.199	a69.01 ±3.196
	2.5	a12.32 ±0.001	a1.98 ±0.014	b34.25 ±0.353	b83.7 ±0.353	ab41.22 ±0.099	a69.03 ±0.813
	5.0	a12.37 ±0.028	a2.00 ±0.011	c32.38 ±0.176	c83.4 ±0.353	ab40.64 ±0.204	a68.04 ±0.537
	10.0	a12.26 ±0.037	a1.98 ±0.009	d30.25 ±0.000	d83.0 ±0.700	b40.07 ±0.502	a67.39 ±2.708
SIMETO	0 (Sound)	a12.42 ±0.032	a1.89 ±0.012	a38.00 ±1.414	a78.9 ±0.000	a49.44 ±0.034	a66.52 ±1.562
	2.5	a12.32 ±0.134	a1.90 ±0.040	ab35.25 ±0.000	b78.7 ±0.353	ab49.12 ±0.110	a65.97 ±0.827
	5.0	a12.34 ±0.011	a1.91 ±0.042	b32.88 ±0.176	c78.6 ±0.353	bc48.66 ±0.275	a64.25 ±1.414
	10.0	a12.35 ±0.037	a1.92 ±0.005	c29.88 ±0.176	d78.3 ±0.000	c48.25 ±0.020	a64.69 ±0.530

No significant differences were detected in grains protein content, ash content and semolina yield between sound samples and damaged samples.

However significant differences were found in SDS-sedimentation test, TW and TKW.

SDS-sedimentation test was significantly lower at all percentages of damaged kernels respect to sound kernels in Iride, while in Simeto at 5% and 10% damaged kernels respect to sound kernels (Table 1). The decrease of SDS-sedimentation test values, respect to sound kernels, range from 7.4% at 2.5% of damaged kernels to 12.5% and 18.2% respectively for 5 % and 10% damaged kernels in Iride. In Simeto, SDS-tests values decreased 13.5% respect to sound kernels, at 5% of damaged kernels. The maximum decrease of SDS-sedimentation test in Simeto was 21.4% at 10% of damaged kernels. SDS-sedimentation test values found in this study agree with the results found in bread wheat (Karaba and Ozan 1998; Kinaci and Kinaci 2004; Vaccino et al. 2006).

TW was significantly lower at all the percentages of damaged kernels respect to sound kernels considering both cultivars (Table 1). TW decreased with the increasing of percentage of damaged kernel respect to sound kernel. The results of TW were similar to those found in bread wheat (Karaba and Ozan 1998; Hariri et al. 2000). TKW was significantly lower at 10% damaged kernels respect to sound kernels, in Iride, and at 5% and 10% damaged kernels in Simeto (Table 1). TKW in Iride decreased 3.6% at 10% of damaged kernel respect to sound kernel while in Simeto decreased 1.6% and 2.4% respectively at 5% and 10% of damaged kernels respect to sound kernels. TKW results recorded in this study agree with the results found in bread wheat (Karaba and Ozan 1998; Kinaci and Kinaci 2004; Vaccino et al. 2006).

Semolina quality

Semolina quality data are reported in tables 2, 3 and 4. Semolina protein content (Table 2) was about 1% less than grains wholemeal (Table 1). No significant difference was detected in semolina protein content between sound and damaged kernels samples considering all the percentages (Table 2). Wheat bugs did not affect gluten quantity obtained from semolina. No significant differences were detected in wet and dry gluten between sound kernels and damaged kernels in both cultivars (Table 2). These results agree with those of Petrova (2002) in durum wheat. The gluten quality was also evaluated using the GI analysis. GI was significantly lower at 10% damaged kernel respect to sound semolina in Iride, and at 5% damaged kernels in Simeto. GI decreased significantly by almost 20% in Iride at 10% damaged kernels respect to sound kernels while in Simeto the loss was about 5% at 5% damaged kernel (Table 2). Olanca et al. (2009), in durum wheat, found a high effect of wheat bugs in wet, dry gluten and GI in samples with 20% damaged kernel.

Table 2. Wheat bugs damaged kernel effects on protein, wet and dry gluten, gluten index (GI), glutograph stretching time and colour (yellow and brown index) are reported. All the parameters were determined in semolina. For each cultivar, data followed by the same letter are not significantly different (P < 0.05). Data are mean ± standard deviation.

SEMOLINA QUALITY CHARACTERISTICS								
CULTIVAR	DAMAGED KERNEL (%)	Protein (%Dry matter)	Wet gluten (%)	Dry gluten (%)	Gluten index (%)	Stretching Time (s)	Yellow index (b*)	Brown index (100-l*)
IRIDE	0 (Sound)	a11.45 ±0.150	a21.31 ±0.385	a8.23 ±0.028	a93.50 ±1.414	a127 ±5.798	a19.83 ±0.191	a9.74 ±0.134
	2.5	a11.43 ±0.073	a21.89 ±1.113	a8.29 ±0.137	a87.50 ±2.828	ab90 ±35.921	a19.77 ±0.453	b9.36 ±0.014
	5.0	a11.31 ±0.154	a21.79 ±0.445	a8.15 ±0.123	ab84.25 ±2.474	ab57 ±15.874	a19.96 ±0.000	ab9.45 ±0.106
	10.0	a11.35 ±0.052	a22.45±0.385	a8.20 ±0.024	b75.00 ±2.828	b24 ±3.111	a19.79 ±0.163	ab9.42 ±0.007
SIMETO	0% (Sound)	a11.49 ±0.125	a20.63±0.130	a8.17 ±0.035	a98.50 ±1.414	a134 ±0.141	a19.23 ±0.219	a10.28 ±0.304
	2.5	a11.45 ±0.027	a21.98 ±1.665	a8.45 ±0.304	ab95.00 ±0.707	a123 ±0.5303	a18.88 ±0.247	a9.82 ±0.275
	5.0	a11.45 ±0.001	a21.87 ±0.979	a8.49 ±0.053	b93.50 ±1.414	a105 ±18.384	a18.79 ±0.042	a9.78 ±0.049
	10.0	a11.48±0.014	a21.25±0.162	a8.32 ±0.173	ab95.25 ±0.353	a76 ±33.163	a18.79 ±0.183	a9.93 ±0.162

The glutograph value stretching time was determined in this study to evaluate gluten quality. Stretching time was significantly lower only in Iride, with a loss of about 80%, at 10% damaged kernel respect to sound semolina (Table 2). Analogous results were found in bread wheat by Fogliazza et al. (2006). As regards semolina colour, it was not affected by wheat bugs, considering all the data, with an exception for brown index in Iride at 2.5% of damaged kernels (Table 2). Koksel et al. (2009) found that wheat bugs affected semolina colour at 40% damaged kernel.

Alveograph values were heavily affected by wheat bugs (Table 3). The dough strength (W), the principal alveographic index, significantly decreased from 2.5% damaged kernels respect to sound kernels and continued decreasing at 5% and 10% damaged kernels, in both cultivars.

Table 3. Wheat bugs damaged kernel effects on alveograph parameters (W, P, L, P/L and G). All the parameters were determined in semolina .For each cultivar, data followed by the same letter are not significantly different (P < 0.05). Data are mean ± standard deviation.

SEMOLINA QUALITY CHARACTERISTICS						
CULTIVAR	DAMAGED KERNEL (%)	Strength W (10^{-4}J)	Tenacity P(mm)	Extensibility L(mm)	Ratio P/L	Swelling Index G (cm^{-3})
IRIDE	0 (Sound)	a193.5 ±7.770	a99.0 ±1.410	a52.5 ±4.949	a1.90 ±0.205	a16.15 ±0.777
	2.5	b129.0 ±4.240	b79.0 ±2.820	ab45.5±0.707	a1.74 ±0.091	ab14.90 ±0.141
	5.0	c91.0 ±4.240	c66.5 ±2.120	bc39.0 ±0.000	a1.71 ±0.0494	bc13.90 ±0.000
	10.0	d47.0 ±2.820	d46.0 ±1.410	c30.5 ±0.707	a1.51 ±0.014	c12.30 ±0.141
SIMETO	0 (Sound)	a197.5 ±2.120	a147.0 ±1.410	a31.5 ±0.707	a4.67 ±0.056	a12.60 ±0.000
	2.5	b138.0 ±1.410	b118.0 ±8.480	a28.5 ±3.530	ab4.19 ±0.820	ab11.90 ±0.707
	5.0	c108.0 ±2.820	c94.5 ±2.120	a28.5 ±0.707	ab3.32 ±0.007	ab11.90 ±0.141
	10.0	d65.0 ±4.240	d68.5 ±3.530	a25.0±0.000	b2.74 ±0.141	b11.10 ±0.000

The loss of W between sound samples and damaged kernels samples was 33.3% and 30.1% respectively for Iride and Simeto at 2.5% damaged kernels. The loss of W increased up to 75.7% and 67.1% respectively for Iride and Simeto at 10% damaged kernels. The loss of W was linked to the decrease of dough tenacity (P), another important alveographic index. P significantly decreased from 2.5% damaged kernels respect to sound kernels in both cultivars. The loss of P was 20.2% and 19.7% respectively for Iride and Simeto at 2.5% damaged kernels respect to sound sample. The loss of P increased up to 46% and 68.5% respectively for Iride and Simeto at 10% damaged kernels. The dough extensibility (L) was the alveographic index less affected by wheat bugs. L was significantly reduced only in Iride at 5% and 10% damaged kernels respect to sound kernels, while in Simeto no significant difference was found. The P/L was more affected in the cultivar Simeto, respect to Iride, depending on the loss of P and the no variation of L. Therefore the ratio P/L was significantly lower only in Simeto at 10% damaged kernels respect to sound kernels. The alveographic index G, as expected, showed a variation similar to that of L. Alveographic indexes W, P, L, P/L and G therefore were affected significantly by wheat bugs. For the first time the alveograph test was performed at different known level of infestation in durum wheat, as it is to our knowledge. The results of alveograph test are similar to those found in bread wheat (Karababa and Ozan 1998; Corbellini et al. 2001; Fogliazza et al. 2006).

According to our knowledge, there is no study reporting information about semolina affected by different percentage of wheat bugs damaged kernels using the Mixolab equipment.

Table 4. Wheat bugs damaged kernel effects on Mixolab parameter water absorption, stability, C1, C2, C3, C4, C5, and the differences C1-C2, C3-C2, C3-C4 and C5-C4. For each cultivar, data followed by the same letter are not significantly different ($P < 0.05$). All the parameters were determined in semolina. Data are mean ± standard deviation.

SEMOLINA QUALITY CHARACTERISTICS								
CULTIVAR	DAMAGED KERNEL (%)	Water Absorption (%)	Stability (min)	C1 (Nm)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
IRIDE	0 (Sound)	a52.5±0.283	a10.59±0.085	a1.12 ±0.282	a0.71 ±0.021	a2.16 ±0.056	a1.78 ±0.007	a3.55 ±0.014
	2.5	a52.3±0.000	b11.31±0.106	a1.10 ±0.000	ab0.66 ±0.007	a2.20 ±0.007	b1.64 ±0.014	b3.35 ±0.028
	5.0	a52.3±0.071	c12.03±0.120	a1.11 ±0.282	bc0.64 ±0.007	ab2.21 ±0.035	b1.66 ±0.042	ab3.45 ±0.014
	10.0	a52.3±0.000	a10.71±0.035	a1.11 ±0.042	c0.59 ±0.021	b2.34 ±0.000	c1.89 ±0.021	c3.74 ±0.042
SIMETO	0 (Sound)	a56.0±0.000	a12.03±0.354	a1.12 ±0.007	a0.78 ±0.007	a2.28 ±0.021	a2.04 ±0.028	a3.29 ±0.063
	2.5	a55.8±0.283	a10.71±0.141	a1.10 ±0.035	a0.73 ±0.028	b2.12 ±0.007	b1.89 ±0.035	b2.95 ±0.106
	5.0	a56.0±0.000	a10.90±0.120	a1.11 ±0.000	a0.71 ±0.007	bc2.08 ±0.014	b1.92 ±0.014	ab3.03 ±0.084
	10.0	a56.2±0.283	b11.47±0.021	a1.11 ±0.035	b0.62 ±0.021	c2.04 ±0.021	ab1.98 ±0.028	ab2.99 ±0.021

CULTIVAR	DAMAGED KERNEL (%)	C1-C2 (Nm)	C3-C2 (Nm)	C3-C4 (Nm)	C5-C4 (Nm)
IRIDE	0 (Sound)	a0.42 ±0.007	a1.46 ±0.077	a0.39 ±0.049	a1.78 ±0.007
	2.5	a0.45 ±0.007	a1.54 ±0.000	b0.56 ±0.021	a1.71 ±0.042
	5.0	ab0.48 ±0.021	ab1.57 ±0.042	b0.55 ±0.007	a1.79 ±0.028
	10.0	b0.53 ±0.021	b1.76 ±0.021	ab0.46 ±0.021	a1.86 ±0.063
SIMETO	0 (Sound)	a0.34 ±0.000	a1.50 ±0.014	a0.24 ±0.007	a1.25 ±0.035
	2.5	a0.37 ±0.007	b1.39 ±0.021	a0.23 ±0.042	a1.06 ±0.141
	5.0	b0.41 ±0.007	b1.38 ±0.007	ab0.16 ±0.028	a1.11 ±0.098
	10.0	a0.42 ±0.007	a1.46 ±0.077	a0.39 ±0.049	a1.78 ±0.007

Mixolab values are reported in table 4. There were no significant differences on water absorption, between sound kernels samples and damaged kernels sample in both cultivars. In Iride the water absorption mean value was 52.3% while in Simeto was 56%. Some significant differences were recorded in stability parameter, but it could be stated that wheat bugs damaged kernels had no effect considering the range of stability.

The parameter C1, as expected, did not show significant difference for any of the percentage of damaged kernels, for both cultivars reaching 1.1 Nm as standard protocol. The parameters C2 and C1-C2 were significantly affected by damaged kernels respect to sound kernels. C2 value is significantly lower at 5% and 10% of damaged kernels respect to sound kernels in Iride and only at 10% of damaged kernels respect to sound kernels in Simeto. C1-C2 value is significantly higher, respect to sound semolina, at 10% of damaged kernels in Iride and at 5% and 10% of damaged kernels in Simeto. The results obtained of C2 and C1-C2 indicated a weakening of protein fraction by damaged kernels respect to sound kernels, agreeing with the results reported for the other tests in semolina. The other parameters C3, C4 and C5 are related with starch characteristics of semolina. C3 value is significantly higher only at 10% damaged kernel respect to sound kernels in Iride, while in Simeto was significantly lower at all percentage of damaged kernel (Table 4). Higher values of C3 (maximum gelatinization peak) indicated a higher consistency and it could depend to the major capacity of water binding. C4 value was significantly lower at 2.5% and 5% damaged kernels respect to sound semolina in both cultivars, but it was significantly higher at 10% damaged kernels in Iride. C5 value was significantly lower at 2.5% of damaged kernels respect to sound semolina in both cultivar but was significantly higher at 10% damaged kernels respect to sound semolina in Iride. The value C3-C2 was significantly higher at 10% of damaged kernels respect to sound semolina in Iride while was significantly lower respect to sound semolina at all damaged kernels percentage in Simeto. The value C3-C4 respect to sound semolina was significantly higher at 2.5% and 5% of damaged kernels in Iride and it was significantly lower at 10% damaged kernels in Simeto. The value C5-C4 was not significantly different in any of the cultivars. The values of C3, C4 C5, and the differences C3-C2, C3-C4, indicated a different and apparently controversial behaviour depending on the cultivar, at different percentage of damaged kernels respect to sound kernels, as shown above. According to the study of Rosell et al. (2002b) in bread wheat, wheat bugs did not alter the amylase activity and did not affect starch. Therefore these contrasting results between Iride and Simeto, as regards starch behaviour, probably depended on the effects of wheat bugs on proteins considering the interactions of starch and proteins (Chedid and Kokini 1992; Eliasson and Tjerneld 1986; D'Egidio et al. 1984) which in turn depend on the starch composition (ratio amylopectin-amylose) and on specific proteins composition (gliadins and glutenins), being all these specific characteristics particular and peculiar of each cultivar.

Pasta cooking quality

As regards pasta cooking quality no significant differences were found between sound pasta and any of the percentage of damaged kernels, in both cultivars, for any of the parameters examined which define the cooking quality of pasta (Table 5).

Table 5. Wheat bugs damaged kernel effects on pasta quality cooking parameters: water absorption, cooking loss, total organic matter (TOM), sensory judgment (SJ) (stickiness, firmness, bulkiness) and the arithmetic mean of the three textural components (Global). For each cultivar, data followed by the same letter are not significantly different ($P < 0.05$). Data are mean ± standard deviation.

SPAGHETTI QUALITY								
					SENSORY JUDGMENT			
CULTIVAR	DAMAGED KERNEL (%)	Water absorption -in cooking- (g)	Cooking loss (g/L)	TOM (%)	Stickiness	Firmness	Bulkiness	Global
IRIDE	0 (Sound)	a183.0 ±10.32	a6.135 ±0.311	a2.3 ±0.219	a34.0 ±1.414	a65 ±0.000	a55 ±0.000	a51.3 ±0.471
	2.5	a168.0 ±0.141	a6.090 ±0.098	a2.0 ±0.237	a43.5 ±2.121	a67 ±0.000	a60 ±0.000	a56.8 ±0.707
	5.0	a172.1 ±13.29	a5.815 ±0.339	a2.1 ±0.347	a43.0 ±7.071	a65 ±0.000	a55 ±7.071	a54.3 ±4.714
	10.0	a169.6 ±7.21	a5.873 ±1.311	a2.7 ±0.050	a33.5 ±2.121	a65 ±0.000	a54 ±1.414	a50.8 ±0.235
SIMETO	0 (Sound)	a177.1 ±7.919	a7.400 ±0.120	a2.46 ±0.442	a38.0 ±7.071	a67.5 ±0.707	a53.5 ±4.949	a53.0 ±3.771
	2.5	a178.3 ±6.363	a6.877 ±0.286	a2.30 ±0.289	a31.5 ±2.121	a68.5 ±2.121	a51.5 ±4.949	a50.5 ±3.064
	5.0	a183.0 ±2.404	a6.920 ±0.254	a2.49±0.311	a24.0 ±5.656	a67.0 ±0.000	a47.0 ±7.071	a46.0 ±4.242
	10.0	a177.4 ±5.798	a7.488 ±0.053	a3.12 ±0.958	a23.0 ±7.071	a62.5 ±0.707	a42.5 ±0.707	a42.7±2.828

It was reported that baking quality was deteriorated from 3-5% of wheat bug damaged kernels (Karababa and Ozan 1998; Hariri et al. 2000; Vaccino et al. 2006) while it was reported that spaghetti quality was affected at >20% wheat bug damaged kernel (Ozderen et al., 2008) although this high level of wheat bugs damaged is not suitable for industry, because a wheat with a >10% of damaged kernel is refused (Koksel et al. 2002). The bread making process is probably more affected than the pasta making process by protease enzyme of wheat bugs. Protease enzymes during bread making (mixing, fermentation) probably had better conditions to act and to make more damage respect pasta making. In the pasta making the semolina is shortly mixed with around 30% of water (respect to 60% water of bread making) extruded at high pressure, and dried at medium high temperature.

In this study is reported that spaghetti quality was not affected by wheat bugs neither at 10% damaged level, although semolina quality is largely affected (Tables 1, 2, 3 and 4). Firstly the protein content, the primary factor influencing pasta quality, was not affected neither at 10% damaged kernel (Table 1 and 2). Moreover it could be explained by the pasta making process, probably mixing time is too short and hydration is too low and it did not allow a large activity of proteolytic enzymes of wheat bugs not affecting pasta quality neither at 10% of damaged kernels. On the other hand spaghetti samples produced with sound semolina, recorded values of TOM > 2.1, indicating a low quality (D'Egidio and Nardi 1996) and considering that the cooking protocol lasted for 13 min (normally an overcooking time), it reduces the differences when comparing pasta of poor quality and it is more difficult to differentiate. A recent study (Bonomi et al. 2012) indicating that pasta with a less compact protein network, probably the case of pasta obtained with wheat bugs damaged kernels, performed better when slightly overcooked, reinforces these results.

CONCLUSIONS

The presence of damaged kernel by wheat bugs affected durum wheat quality, as regard physical chemical grain characteristic decreasing the values of SDS-test, TW and TKW. The reduction of quality in many cases is already from 2.5% wheat bugs damaged kernels. On the other hand, protein, ashes and semolina yield were not affected by wheat bugs, even at 10% damaged kernel, the maximum damaged percentage considered in this study.

Semolina quality decreased and it was evident particularly considering the alveographic parameters W and P, which were heavily reduced from 2.5%

wheat bugs damaged kernels. GI and stretching time determined by glutograph was reduced but from 5% wheat bugs damaged kernels.

Mixolab parameters linked with semolina protein quality (C2, C1-C2) indicate a weakening of protein from 5% wheat bugs damaged kernels. The others Mixolab parameters linked with starch behaviour were affected but in a controversial way depending on the cultivar.

Spaghetti quality was not reduced by wheat bugs damaged kernels, considering water absorption, cooking loss, TOM and SJ. Taking in account the important effect of wheat bugs damaged kernels on semolina quality it was expected a quality deterioration of spaghetti but no evidence was found. Technological process of making pasta probably masks the detrimental effect of wheat bugs on durum wheat.

Further researches on the effects of wheat bugs in spaghetti quality would be useful, considering the same percentage of wheat bug damaged kernels, but testing a wider range of different cultivars of durum wheat and using the optimal cooking time, corresponding to the disappearance of starchy central core of spaghetti. On the other hand, the use of semolina for baking traditional types of bread in the Mediterranean countries is very common and therefore studies about the effects of wheat bugs in durum wheat bread-making are suggested.

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DISCUSIÓN Y RESULTADOS

LAS CHINCHES DE LOS CEREALES

En el primer capítulo de la tesis se estudiaron las chinches de los cereales en el trigo duro de Cerdeña (Italia), que es uno de los cultivos más importantes de la isla, como también ocurre en el resto de Italia.

En las campañas de prospección en campos de trigo duro de Cerdeña se recogieron 867 individuos y se encontraron cuatro especies diferentes de chinches de cereales pertenecientes a los géneros *Eurygaster* y *Aelia*, siendo más abundante *Eurygaster* spp. que *Aelia* spp. La especie predominante fue *Eurygaster austriaca*, seguida por *Aelia germari*, *Eurygaster maura* y *Aelia acuminata*. No se encontraron otras especies de chinches de cereales citadas en Cerdeña (*E. hottentotta*, *A. rostrata*, *A. notata* y *A. klugii*) o en otras parte de Italia (*A. sibirica*, *E. testudinaria*, *E. dilaticollis* y *E. integriceps*), según consta en diversas publicaciones (Servadei 1952; Tamanini 1988; Faraci y Rizzotti Vlach 1995; Derjanschi and Péricart 2005; Fauna Europaea 2011)

Las cuatro especies presentes se han encontrados en general en las tres zonas de muestreo, representativas del cultivo de trigo duro en la isla, con la única excepción de *A. acuminata*, que no se encontró en la Zona 1.

En la mayoría de los campos la densidad de chinches fue bastante baja, inferior a 1 individuo/m^2. La densidad registrada fue mayor en junio respecto a mayo, con 0,5-2 individuos/m^2 en aproximadamente el 50% de los campo. De todas formas hubo alguna excepción y en unos campos de la Zona 2 se registraron densidades de 4,3 - 7,3 y 8,2 individuos/m^2 superiores al umbral de daño de 4 individuos/m^2 establecido por Paulian y Popov (1980).

En mayo, en los campos de trigo, se encontraron pocos adultos (<1 individuos/m^2) y ninguna ninfa. Estas observaciones son coherentes con el ciclo biológico de las chinches de los cereales, que hibernan en diversos refugios (piedras, hojas secas, matas de hierba) hasta que se trasladan a los campos de cereales para alimentarse y aparearse. En mayo la actividad reproductiva todavía no ha comenzado. Es importante conocer la densidad de los adultos que han hibernado y que llegan a los campos de trigo, porque está asociada a la densidad de ninfas y a la nueva generación de adultos (Kutuk *et al.*, 2010), que es la más perjudicial para la cosecha de trigo.

En junio, cuando el trigo estaba en la fase final del llenado del grano-maduración, los adultos hibernantes ya se habían reproducido y se encontró una población mixta de ninfas y adultos, determinando una densidad de población más alta con respecto a mayo, como se explicó anteriormente. Nuestros resultados, en cuanto a la densidad de chinches de los cereales en mayo y junio, concuerdan con otros estudios como el de Hariri et al. (2000) en Siria, Popov et al. (2003) en Rumania, Kutuk et al. (2010) y Canhilal et al. (2005) en Turquía.

Las densidades de población varían considerablemente entre las tres zonas de muestreo. La densidad media en la Zona 2 fue significativamente mayor que en la Zona 3 y en la Zona 1. Hay muchos factores que influyen en las condiciones óptimas para el desarrollo de las chinches de los cereales, como las condiciones climáticas, las zonas cultivadas, los parásitos y los sitios de hibernación (Kutuk et al. 2010; Popov et al. 2003; Javahery 1996), pudiendo determinar diferentes densidades de población. Las temperaturas más altas estimulan el desarrollo de las chinches de los cereales (Iranipour et al. 2010; Javahery 1996). Durante el período 20 abril - 20 junio, dentro del cual se efectuaron los dos muestreos, la temperatura media en la Zona 2 fue la más alta, y en la Zona 1 fue la más baja, en ambos años de estudio. Estas diferencias térmicas podrían explicar las diferentes densidades en las tres zonas.

Considerando los datos en su conjunto, no se encontraron diferencias significativas en las densidades entre el borde y el interior del campo. Una posible explicación es el hecho que muchos campos incluidos en el muestreo estaban justo a lados de otros campos en un continuo y sin un verdadero borde que limitara adecuadamente un campo de otro. Por el contrario, en otros estudios en el que los campos de trigo fueron muestreados en el borde y en el interior, se encontraron diferencias entre las zonas del campo. En una región del Sur de Rusia, por ejemplo, *E. integriceps* empezó a colonizar el campo a partir de sus bordes; sin embargo, las siguientes generaciones fueron más abundantes en el centro del campo (Afonina et al. 2001). En el noreste de España las diferencias entre la densidad en el borde y el interior del campo se encontraron sólo para *Aelia* spp. pero no para *Eurygaster* spp. (Pérez-Rodríguez et al. 2008). Es importante conocer la distribución de las chinches de los cereales en el campo con el fin de seleccionar el método de muestreo más apropiado. Esto podría permitir, además, la detección temprana de las chinches del trigo, antes de la cópula y la puesta, que es muy importante para el control de esta plaga.

EFECTOS EN LAS PROTEÍNAS, GLIADINAS Y GLUTENINAS

En el segundo capítulo investigamos cómo la presencia de granos picados por las chinches de los cereales afectaba a las prolaminas, gliadinas y gluteninas, del trigo duro. La alteración de las gluteninas y de las gliadinas fue evidente, con resultados similares a los descritos en trigo blando, aunque en trigo blando sólo se hicieron análisis a alta temperatura (Sivri et al. 1998, 1999; Rosell et al. 2002).

Diversos estudios han investigado el efecto de las chinches de los cereales sobre las proteínas del trigo blando. Sivri et al. (1999) estudiaron el efecto en muestras de trigo blando con un 33% de granos dañados por *E. maura*, porcentajes similares a los utilizados en el presente estudio. Utilizaron la determinación con RP-HPLC y hallaron que más del 80% de las gluteninas se degradaba después de 30 min de incubación a 37ºC. El aumento de la degradación tras 1h y 2h de incubación fue muy limitado. En otro estudio, también sobre trigo blando, se reportó con el análisis de la FZCE, una disminución de las fracciones de gluteninas HMW-GS y LMW-GS (Rosell et al. 2002).

Nuestras observaciones indican que, en las condiciones del estudio (30% de granos picados) las gluteninas del trigo duro se degradan rápidamente, con independencia de la temperatura de incubación. Esta rápida despolimerización de las gluteninas probablemente debida a una reducción de los enlaces disulfuro entre cadenas, sería compatible con el marcado aumento de los grupos tiólicos libres registrado por Pérez et al. (2005) durante la degradación. Ante estos resultados, cabe plantearse la posibilidad de que la degradación de las gluteninas no sea debida sólo a la actividad de las proteasas de las chinches de los cereales, sino también a algún otro factor ligado a la actividad alimenticia de estos insectos que actuaría como un reductor, todavía por determinar.

En cuanto a las gliadinas, se observó un aumento inicial en su concentración en el presente estudio, probablemente debido a la presencia de los productos de la degradación de las gluteninas, que tienen una más alta movilidad electroforética, como describen Rosell et al. (2002) en trigo blando.

En otro estudio (Sivri et al. 1998) sobre las gliadinas de trigo blando dañado por las chinches de los cereales con el análisis A-PAGE, se evidenció una disminución de algunas nuevas bandas aparecidas, así como de las bandas originales de gliadinas; esta disminución fue más evidente al aumentar los tiempos de incubación (120 min y 240 min), y es compatible con los resultados encontrados en este estudio para el trigo duro.

Los resultados obtenidos en trigo duro dañado por las chinches de cereales indican que la alteración de las gliadinas depende de la temperatura y del tiempo, mostrando una degradación general de las gliadinas y una degradación especifica de las gluteninas solubilizadas.

Estos resultados obtenidos en trigo duro, podrían explicar la modificación de las características de la calidad de la pasta "spaghetti" cuando la sémola de trigo dañada (>20% trigo dañado) por las chinches de los cereales se utiliza para hacer pasta. En esta sémola se produce una pérdida de la calidad del gluten, de la calidad de cocción y una disminución de las propiedades sensoriales (Ozderen et al. 2008; Köksel et al. 2009).

Podrían esperarse consecuencias similares en la pérdida de la calidad panificadora cuando se producen ciertos panes tradicionales de la cuenca del Mediterráneo hechos con sémola obtenida a partir de trigo duro dañado por las chinches de los cereales, aunque todavía no se ha realizado ningún estudio para evaluar esta hipótesis.

EFECTOS EN LA PASTA

Finalmente, en el Capítulo 3, se estudian los efectos de las chinches de los cereales en el producto más importante que se obtiene del trigo duro que es la pasta. Las variedades de trigo duro seleccionadas para este estudio fueron Iride y Simeto por ser unas de las más cultivadas en Italia. Se evaluaron las características cualitativas de los granos y de la sémola con la cual se elaboraría la pasta, y por supuesto las características cualitativas de la pasta así obtenida.

Por lo que concierne a las características físico-químicas de los granos, se encontraron diferencias significativas entre la muestra obtenida con granos sanos respecto a las muestras obtenidas con granos dañados en la prueba de sedimentación SDS, en el peso hectolítrico y en el peso de mil granos, dependiendo del cultivar (Iride y Simeto) y del porcentaje de granos dañados (2,5%, 5% y 10%). Los resultados de SDS-sedimentación encontrados en este estudio concuerdan con los resultados encontrados en trigo blando (Karaba y Ozan 1998; Kinaci y Kinaci 2004; Vaccino et al. 2006). El peso hectolítrico fue significativamente menor en todos los porcentajes de granos dañados respecto a los sanos en ambos cultivares y disminuyó con el aumento del porcentaje de granos dañados. Los resultados de peso hectolítrico eran similares a los registrados en el trigo blando (Karaba y Ozan 1998; Hariri et al. 2000). El peso de mil granos fue significativamente menor respecto a los granos sanos, dependiendo de la cultivar y del porcentaje de granos dañados. Nuevamente los resultados del peso de mil granos de este estudio fueron similares a los resultados encontrados en trigo blando (Karaba y Ozan 1998; Kinaci y Kinaci 2004; Vaccino et al. 2006).

Por lo que concierne la calidad de la sémola, las chinches de los cereales no afectaron la cantidad de gluten obtenido a partir de sémola. No se detectaron diferencias significativas en el gluten húmedo y seco entre las muestras de sémola de granos sanos y de granos dañados en ninguno de los cultivares. Estos resultados concuerdan con los de Petrova (2002). El índice de gluten (IG) fue significativamente inferior con respecto al control dependiendo del cultivar y del porcentaje de granos dañados. Olanca et al. (2009) encontraron un fuerte efecto de las chinches de los cereales en el gluten húmedo, seco e IG en muestras de trigo duro con más del 20% de granos dañado. El tiempo de relajación determinado con el glutógrafo fue significativamente inferior sólo en Iride con el 10% de granos dañados, perdiendo un 80% del valor respecto a la sémola control. Resultados análogos fueron hallados en trigo blando (Fogliazza et al. 2006).

El color de la sémola, considerando los datos en su conjunto, no fue afectado por las chinches de los cereales. Koksel et al. (2009) encontraron que las chinches del trigo afectan el color de la sémola cuando el nivel de granos dañados alcanzaba el 40%.

Por lo que sabemos, esta es la primera vez que se realiza la prueba alveográfica en trigo duro a diferentes niveles conocidos de granos dañados por las chinches de los cereales. Los parámetros alveográficos fueron afectados significativamente de forma importante ya a partir de 2,5% de granos dañados. Los índices alveográficos W, P, L, P/L y G, se vieron afectados significativamente por las chinches del trigo, en particular hubo una fuerte pérdida de P y consecuentemente de W. Los resultados obtenidos con el alveógrafo son similares a los encontrados en el trigo blando (Karababa y Ozan 1998; Corbellini et al. 2001; Fogliazza et al. 2006).

Según nuestro conocimiento, no hay información sobre el estudio de sémola afectada por diferentes porcentajes de granos dañados por las chinches de los cereales utilizando el equipo Mixolab, ni en trigo blando ni en trigo duro.

No se registraron diferencias significativas en el valor de absorción. Se registraron algunas diferencias significativas en el parámetro de estabilidad, según el porcentaje de daño, pero se podría decir que los granos dañados por las chinches de los cereales no tuvieron efecto en la calidad de la sémola teniendo en cuenta el rango de estabilidad.

El parámetro C1, como se esperaba, no mostró ninguna diferencia significativa para ningún porcentaje de granos dañados, para ambos cultivares, llegando a 1,1 Nm como dicta el protocolo estándar. Los parámetros C2 y C1-C2 se vieron afectados significativamente por los granos dañados respecto a los granos sanos. Los resultados obtenidos de C2 y C1-C2 indican un debilitamiento de la fracción proteica en las muestras dañadas respecto a las muestras sanas, de acuerdo con los resultados reportados para los otros análisis de la sémola.

Los parámetros C3, C4 y C5, y las diferencias C3-C2, C3-C4, C5-C4, están relacionados con las características del almidón de la sémola. Su comportamiento fue diferente y aparentemente controvertido dependiendo del cultivar, en diferentes porcentajes de granos dañados. De acuerdo con el estudio de Rosell et al. (2002) en trigo blando, las chinches de los cereales no alteraban la actividad amilásica y no afectaban el almidón. Por lo tanto estos resultados aparentemente contradictorios entre Iride y Simeto, en cuanto al comportamiento del almidón, probablemente dependen de los efectos de las chinches de los cereales en las proteínas teniendo en cuenta las interacciones de almidón y proteínas (Chedid y Kokini 1992; Eliasson y Tjerneld 1990; D'Egidio et al. 1984). Estas interacciones dependen a su vez de la composición del almidón (amilosa-amilopectina) y de la composición específica de la fracción proteica (gliadinas y gluteninas), siendo todas estas características específicas y peculiares de cada cultivar.

En cuanto a la calidad de cocción de la pasta, no se encontraron diferencias significativas entre la pasta obtenida con muestras sanas y la obtenida con muestras dañadas.

Se reportó que la calidad panificadora del trigo blando se deteriora con un 3-5% de granos dañados por la chinches de los cereales (Karababa y Ozan 1998; Hariri et al. 2000; Vaccino et al. 2006). Se conoce que la calidad de cocción de los “spaghetti” se vio afectada cuando la proporción de granos dañados por las chinches de los cereales era superior al 20% (Ozderen et al. 2008), aunque este alto nivel de granos dañados por las chinches de los cereales no es utilizable por la industria, que rechaza un trigo con un porcentaje de granos dañados superior al 10% de granos dañados (Koksel et al. 2002).

El proceso de elaboración del pan se ve probablemente más afectado por las proteasas de las chinches de los cereales que el proceso de producción de la pasta. Durante algunas fases de la elaboración del pan (amasado y fermentación) las proteasas probablemente tienen mejores condiciones de tiempo e hidratación para actuar y determinar un mayor daño respecto al proceso de fabricación de la pasta.

En el proceso de fabricación de la pasta la sémola se mezcla durante un tiempo breve comparado con el amasado y la fermentación del pan. Además la proporción de agua que se utiliza (alrededor de un 30% de agua respecto al 60% de agua de la elaboración del pan) por un lado y el proceso de extrusión a alta presión y el proceso de secado final a temperaturas medio-altas, por otro, hacen probablemente que las proteasas no determinen un daño tan evidente en la pasta.

Nuestros resultados indican que la calidad de los "spaghetti" no se vio afectada por las chinches de los cereales ni al nivel más alto de granos dañados, del 10%, aunque la calidad de la sémola se ve afectada de forma importante. En primer lugar, el contenido de proteína, el factor principal que determina la calidad de la pasta, no se vio afectó ni al 10% de granos dañados. Además esto podría explicarse por el proceso de fabricación de la pasta: probablemente el tiempo de mezcla es demasiado corto y la hidratación es baja no permitiendo una gran actividad de los enzimas proteolíticos de las chinches de los cereales. Como resultado la calidad de la pasta no se ve afectada ni siquiera al 10% de granos dañados.

Es importante señalar que la pasta producida con muestras sanas tenía valores de TOM>2,1 indicando una baja calidad (D'Egidio y Nardi 1996). Además el protocolo estándar de cocción utilizado duraba 13 min, un tiempo de sobre-cocción para los "spaghetti", que se utiliza para evaluar la resistencia de la pasta a la sobre cocción. Un estudio reciente (Bonomi et al. 2012) indica que la pasta con una red de proteínas menos compacta, probablemente el caso de las pastas obtenidas con granos dañados por las chinches de los cereales, da un resultado mejor cuando se sobrepasa de cocción. Estas observaciones son coherentes con nuestros resultados, donde la baja calidad de la pasta control (sin granos dañados) y el exceso de cocción convergen de modo que se hace difícil diferenciar entre la pasta obtenida a partir de muestras sanas y de muestras dañadas.

CONCLUSIONES

CONCLUSIONES

1 – Se detecta en Cerdeña la presencia de chinches de los cereales en el cultivo de trigo duro, siendo 4 las especies encontradas: *E. austriaca*, *A. germari*, *E. maura* y *A. acuminata*.

2 – Dado que en algunos campos, las densidades de los chinches de los cereales sobrepasó el umbral de daño sería aconsejable un seguimiento para la evaluación y control razonado de la plaga.

3 – La actividad alimenticia de las chinches de los cereales tiene una fuerte influencia sobre las proteínas del trigo duro predominantemente sobre la gluteninas. Las gluteninas se despolimerizan rápidamente y casi en toda su totalidad de forma independiente de la temperatura. Las gliadinas se degradan con menor intensidad y de forma dependiente de la temperatura y del tiempo.

4 – La presencia de granos dañados por las chinches de los cereales afecta a la calidad del trigo duro de forma importante ya a partir del 2,5% de granos dañados.

5 – La presencia de granos dañados por las chinches de los cereales, ya a partir del 2,5%, afecta significativamente y de forma negativa al test SDS de sedimentación, del peso hectolítrico y del peso de mil granos, parámetros que determinan las características físico-químicas del grano de trigo duro.

6 – La presencia de granos dañados por las chinches de los cereales afecta a la calidad reológica de las sémolas. Un 2,5 % de granos picados disminuye ya de forma significativa la tenacidad (P) y la fuerza (W) en el ensayo alveográfico. A partir de un 5% se observa un debilitamiento de la fracción proteica en el ensayo del Mixolab.

7 – La calidad de la pasta alimenticia ("spaghetti"), considerando la absorción de agua en cocción, la pérdida en cocción, la materia orgánica total (TOM) y la evaluación sensorial, no se vio perjudicada por la presencia de granos picados por las chinches de los cereales.

SUMMARY AND CONCLUSIONS

INTRODUCTION

The names "sunn pest" and "wheat bug" refer to different species in the genera *Eurygaster* (Hemiptera: Scutellaridae) and *Aelia* (Hemiptera: Pentatomidae). Wheat bugs are widely distributed in various areas of Europe, Asia, and North Africa (Paulian and Popov 1980). The economic importance of wheat bug damage is due to crop losses and/or quality loss of wheat (Kinaci and Kinaci 2004), semolina (Ozderen et al. 2008; Köksel et al. 2009; Salis et al. 2010), or flour (Hariri et al. 2000; Sivri et al. 1999, 2004; Aja et al. 2004; Vaccino et al. 2006; Werteker and Kramreither 2008). The feeding activity of wheat bugs also heavily affects the germination percentage of wheat (Bin et al. 2006). Both nymphs and adults of *Eurygaster* spp. and *Aelia* spp. cause a reduction of wheat quality when they insert their piercing-sucking mouthparts in the kernels and extract the substances within. Feeding activity of wheat bugs involves the injection of proteases in wheat grains. The detrimental effect of such proteases on baking quality is very high, even when only 3–5% of kernels are damaged, and dramatically increases for values higher than 10% (Karababa and Ozan 1998; Hariri et al. 2000).

Durum wheat (*Triticum turgidum* L. var *durum*) is one of the most important cereal crops. The world production is about 35 million tonnes and Italy is one of the most important producers, generating around 15% of the world production (Taylor and Woo, 2011). Durum wheat is the preferred raw material for the manufacture of superior quality pasta (Feillet and Dexter 1996). The main use of durum wheat is for pasta making, but other traditional foods in the Mediterranean countries are also produced as couscous, bulgur and traditional types of bread (Quaglia 1988). The technological quality of durum wheat therefore depends on semolina yield and on semolina quality, the ability of semolina to be processed into pasta that meets the requirements of the usual consumers (Cubadda 1988). There is a universal agreement that protein content is the primary factor influencing pasta quality and that gluten strength is an important secondary factor (D'Egidio et al. 1990; Novaro et al. 1993;

Feillet and Dexter 1996). Most pasta manufacturers demand a minimum semolina protein content (Feillet and Dexter 1996).

There are quite studies related to wheat bugs infesting bread wheat fields and their effects on gluten proteins, physical and rheological properties and baking quality of bread wheat. On the other hand, few studies regarding wheat bugs infesting durum wheat fields and their effects on durum wheat were reported.

OBJECTIVES

In this thesis, we study the presence of wheat bugs in durum wheat field in Sardinia, and the influence of this pest on durum wheat protein fraction, particularly in gliadin and glutenin, and the behavior of the semolina and pasta obtained from durum wheat samples with different percentages of wheat bug damaged grains.

Main Objective

The main objective of this work was to study the effects of wheat bugs in durum wheat (*Triticum turgidum* L. var *durum*) to different levels: in the field, at macromelocular level (protein) and in the final product (semolina and pasta).

Specific Objectives

The specific objectives are stated for each chapters of the thesis:

• Determine species, distribution and density of wheat bugs present in the durum wheat fields of Sardinia in order to know the incidence of plague in the island. (Chapter 1)

• Investigate the effects of wheat bugs at macromolecular level, studying the effects on gluten proteins, gliadin and glutenin. (Chapter 2)

• Evaluate the effects of wheat bugs on grains, semolina and pasta quality of durum wheat. (Chapter 3)

RESULTS AND DISCUSSION

Wheat bugs

During the two years of sampling (2006 and 2007) in the 13 durum wheat fields of Sardinia, two genera, *Eurygaster* and *Aelia*, and four species, *E. austriaca* (Schrank 1776), *E. maura* (Linnaeus 1758), *A. germari* Küster (1852), and *A. acuminata* (Linnaeus 1758), were identified. The most abundant species was *E. austriaca*, which represented 75.1% of the total number of adults collected, followed by *A. germari* (13.6%), *E. maura* (7.1%), and *A. acuminata* (4.2%). These four species were present in all sampling zones (Zone 1, Zone 2 and Zone 3) except for *A. acuminata*, which was never found in Zone 1.

The fields sampled registered a very low density of wheat bugs, between 0 and 1 individuals/m^2. In June in both years, the density of wheat bugs was higher compared to May, and approximately half of the fields registered a density that ranged from 0.5 to 2 individuals/m^2. There were very few fields with densities above 4 individuals/m^2, the density level established as the damage threshold by Paulian and Popov (1980). The exceptions were observed in two fields in Zone 2, which had 4.3 - 7.3 individuals/m^2 and 8.2 individuals/m^2 respectively.

According to the wheat bugs' life-cycle, they overwinter in or under diverse shelters (stones, dry leaves, grass clumps) until they move to cereal fields to feed and mate. Few adults were found in wheat fields in May (< 1 individuals/m^2). No nymphs were found in May, as reproductive activity had not yet begun. It is important to know the density of the overwintered adults in wheat fields because it is associated to nymphs and new-adults generation (Kutuk et al. 2010), which is the most detrimental to wheat crop. In June, when wheat was at the end of grain-filling and maturation, overwintered adults had already reproduced and a mixed population of nymphs and adults was found, resulting in a higher population density with respect to May, as explained above. These findings, regarding wheat bugs densities in May and June, agree with other studies, such as Hariri et al. (2000) in Syria, Popov et al. (2003) in Romania, and Kutuk et al. (2010) and Canhilal et al. (2005) in Turkey.

Population densities varied significantly between zones. The average density in Zone 2 was significantly higher than Zone 3 and Zone 1. Many factors influence the optimal conditions for the development of wheat bugs, such as climatic conditions, areas cropped, parasites, and overwintering sites (Javahery 1996; Popov et al. 2003; Kutuk et al. 2010), and they can determine different population densities. During the period 20 April – 20 June, immediately before the first sampling (1–10 May) until the end of the second sampling (10–20 June), the average temperature in Zone 2 was the highest (in 2007: Zone 2, 19.3º C; Zone 3, 18.7º C; Zone 1, 18.5º C; in 2008: Zone 2, 18.7º C; Zone 3, 18º C; Zone 1, 17.5º C). In both years, during the period considered above, the average maximum temperature was highest in Zone 2, whereas Zone 1 always presented the lowest average minimum temperature. Higher temperatures stimulate the development of wheat bugs (Javahery 1996; Iranipour et al. 2010) and could explain the different densities in the three zones.

Considering the data as a whole, no significant differences in density between the edge and the interior of the field were found. This result was possibly due to the fact that many sampled fields were side by side in a continuum without a properly limiting edge. Conversely, in other studies (Afonina et al. 2001; Pérez-Rodriguez et al. 2008) in which the wheat fields were sampled in the edge and in the interior, differences were found between the field zones. In a region of South Russia, for example, *E. integriceps* began to colonize the field from its edges; however, the following generations were more abundant in the center of the field (Afonina et al. 2001). In northeast Spain, differences between density in the edge and the interior of the field were found only for *Aelia* spp., but not for *Eurygaster* spp. (Pérez-Rodriguez et al. 2008). It is important to know the distribution of the wheat bugs in fields in order to select the appropriate sampling method to be used. This could, moreover, permit the early detection of the wheat bugs before copulation and oviposition, which is very important for the control of this pest.

Effects on proteins, gliadins and glutenins

Total glutenins and gliadins alteration, due to the presence of bug-damaged kernels, was quantified and results were similar to those described in bread wheat (analysis performed only at high temperature). Glutenin results were very much alike to those obtained in bread wheat (Sivri et al. 1998, 1999; Rosell et al. 2002). In wheat cultivated in Turkey and manually infested by *E. maura* bugs, it was seen that after 30 min of incubation, more than 80% glutenin was degraded, and increase of degradation was very low 1 h and 2 h after incubation (Sivri et al. 1999). In that research, the proportions (2:1) of sound and damaged wheat in the blend was similar to that used in the present study, temperature of incubation was 37°C, and determination of reduced glutenins was done by RP-HPLC (Reverse Phase – High Performance Liquid Chromatography).

In bug-damaged bread wheat cultivated in Spain, a decrease of the glutenin fractions HMW-GS (High Molecular Weight Glutenin Subunits) and LMW-GS (Low Molecular Weight Glutenin Subunits) determined with FZCE (Free Zone Capillary Electrophoresis) was also reported (Rosell et al. 2002). Regarding bug-damaged wheat, less information is available on gliadins than on glutenins. The initial increase in the area of gliadins observed in this study, probably due to the presence of glutenins degradation products with a higher electrophoretic mobility, was also described in bug-damaged bread wheat, as in the case of Spanish bread wheat samples and using FZCE (Rosell et al. 2002). In gliadins from bread wheat from Turkey incubated at 37°C, and analysed by A-PAGE (Acid-Polyacrylamide gel electrophoresis), a decrease was also stated, concerning some new bands as well as the original gliadin bands; as in the case of durum wheat, the changes were more obvious with increasing incubation times and most of the gliadins bands were lost after 120 or 240 min of incubation (Sivri et al. 1998). In bug-damaged bread wheat a marked increase of free thiol groups during initial incubation was described (Pérez et al. 2005). Results in durum wheat damaged by wheat bugs indicate that the alteration of the gliadins and glutenins initially begins with a fast depolymerisation of glutenins, independently of temperature, presumably due to a reduction of inter-chain disulphide bonds. The alteration continues, depending on the temperature, showing a general and specific degradation of gliadins and solubilised glutenins. Therefore, degradation of glutenins was possibly due not only to the action of protease enzymes, but also to another not yet determined salivary agent(s), that would act as a reductor. These durum wheat results could explain the modification of pasta (spaghetti) quality characteristics, referring to a loss of gluten quality, cooking values and a deterioration of sensory properties, when semolina from bug-damaged wheat is used to make pasta (Ozderen et al. 2008; Köksel et al. 2009).

Effects on pasta quality

No significant differences were detected in grains protein content, ash content and semolina yield between sound samples and damaged samples. However significant differences were found in SDS-sedimentation test, test weight (TW) and thousand kernels weight (TKW). SDS-sedimentation test was significantly lower at all percentages of damaged kernels respect to sound kernels in Iride, while in Simeto at 5% and 10% damaged kernels respect to sound kernels. The decrease of SDS-sedimentation test values, respect to sound kernels, range from 7.4% at 2.5% of damaged kernels to 12.5% and 18.2% respectively for 5 % and 10% damaged kernels in Iride. In Simeto, SDS-tests values decreased 13.5% respect to sound kernels, at 5% of damaged kernels. The maximum decrease of SDS sedimentation test in Simeto was 21.4% at 10% of damaged kernels. SDS sedimentation test values found in this study agree with the results found inbread wheat (Karaba and Ozan 1998; Kinaci and Kinaci 2004; Vaccino et al. 2006).

TW was significantly lower at all the percentages of damaged kernels respect to sound kernels considering both cultivars. TW decreased with the increasing of percentage of damaged kernel respect to sound kernel. The results of TW were similar to those found in bread wheat (Karaba and Ozan 1998; Hariri et al. 2000). TKW was significantly lower at 10% damaged kernels respect to sound kernels, in Iride, and at 5% and 10% damaged kernels in Simeto. TKW in Iride decreased 3.6% at 10% of damaged kernel respect to sound kernel while in Simeto decreased 1.6% and 2.4% respectively at 5% and 10% of damaged kernels respect to sound kernels. TKW results recorded in this study agree with the results found in bread wheat (Karaba and Ozan 1998; Kinaci and Kinaci 2004; Vaccino et al. 2006).

No significant difference was detected in semolina protein content between sound and damaged kernels samples considering all the percentages.

Wheat bugs did not affect gluten quantity obtained from semolina. No significant differences were detected in wet and dry gluten between sound kernels and damaged kernels in both cultivars. These results agree with those of Petrova (2002) in durum wheat. The gluten quality was also evaluated using the gluten index (GI) analysis. GI was significantly lower at 10% damaged kernel respect to sound semolina in Iride, and at 5% damaged kernels in Simeto. GI decreased significantly by almost 20% in Iride at 10% damaged kernels respect to sound kernels while in Simeto the loss was about 5% at 5% damaged kernel. Olanca et al. (2009), in durum wheat, found a high effect of wheat bugs in wet, dry gluten and GI in samples with 20% damaged kernel.

Alveograph values were heavily affected by wheat bugs. The dough strength (W), the principal alveographic index, significantly decreased from 2.5% damaged kernels respect to sound kernels and continued decreasing at 5% and 10% damaged kernels, in both cultivars. For the first time the alveograph test was performed at different known level of infestation in durum wheat, as it is to our knowledge. The results of alveograph test are similar to those found in bread wheat (Karababa and Ozan 1998; Corbellini et al. 2001; Fogliazza et al. 2006).

According to our knowledge, there is no study reporting information about semolina affected by different percentage of wheat bugs damaged kernels using the Mixolab equipment.

There were no significant differences on water absorption, between sound kernels samples and damaged kernels sample in both cultivars.

The parameter C1, as expected, did not show significant difference for any of the percentage of damaged kernels, for both cultivars reaching 1.1 Nm as standard protocol. The parameters C2 and C1-C2 were significantly affected by damaged kernels respect to sound kernels. C2 value is significantly lower at 5% and 10% of damaged kernels respect to sound kernels in Iride and only at 10% of damaged kernels respect to sound kernels in Simeto. C1-C2 value is significantly higher, respect to sound semolina, at 10% of damaged kernels in Iride and at 5% and 10% of damaged kernels in Simeto. The results obtained of C2 and C1-C2 indicated a weakening of protein fraction by damaged kernels respect to sound kernels, agreeing with the results reported for the other tests in semolina.

As regards pasta cooking quality no significant differences were found between sound pasta and any of the percentage of damaged kernels, in both cultivars, for any of the parameters examined which define the cooking quality of pasta.

It was reported that baking quality was deteriorated from 3-5% of wheat bugs damaged kernels (Karababa and Ozan 1998; Hariri et al. 2000; Vaccino et al. 2006) while it was reported that spaghetti quality was affected at >20% wheat bug damaged kernel (Ozderen et al., 2008) although this high level of wheat bugs damaged is not suitable for industry, because a wheat with a >10% of damaged kernel is refused (Koksel et al. 2002). The bread making process is probably more affected than the pasta making process by protease enzyme of wheat bugs. Protease enzymes during bread making (mixing, fermentation) probably had better conditions to act and to make more damage respect pasta making. In the pasta making the semolina is shortly mixed with around 30% of water (respect to 60% water of bread making) extruded at high pressure, and dried at medium high temperature.

In this study is reported that spaghetti quality was not affected by wheat bugs neither at 10% damaged level, although semolina quality is largely affected. Firstly the protein content, the primary factor influencing pasta quality, was not affected neither at 10% damaged kernel. Moreover it could be explained by the pasta making process, probably mixing time is too short and hydration is too low and it did not allow a large activity of proteolytic enzymes of wheat bugs not affecting pasta quality neither at 10% of damaged kernels. On the other hand spaghetti samples produced with sound semolina, recorded values of TOM > 2.1, indicating a low quality (D'Egidio and Nardi 1996) and considering that the cooking protocol lasted for 13 min (normally an overcooking time), it reduces the differences when comparing pasta of poor quality and it is more difficult to differentiate. A recent study (Bonomi et al. 2012) indicating that pasta with a less compact protein network, probably the case of pasta obtained with wheat bugs damaged kernels, performed better when slightly overcooked, reinforces these results.

CONCLUSIONS

1 – The presence of wheat bugs is detected in Sardinia durum wheat fields. Four species were found: *E. austriaca*, *E. maura* L., *A. germari*, and *A. acuminata* .

2 – As in some fields, the damage threshold by wheat bugs was exceeded, it would be advisable to monitor the pest in order to assess an efficient control.

3 – Wheat bugs feeding activity has a strong influence on durum wheat proteins, predominantly on the glutenin fraction. Glutenins depolymerize rapidly and almost completely independently of temperature. Gliadins degrade less intensively, and their degradation depends on temperature and time.

4 – Wheat bugs damaged grains affect durum wheat quality significantly from 2.5% damaged kernels samples.

5 – The presence of wheat bugs damaged kernels from 2.5% significantly affects the SDS sedimentation test, test weight and thousand grain weight. Those parameters determine the physicochemical characteristics of durum wheat grain.

6 – The presence of damaged grain by wheat bugs affects semolina rheological quality. The alveographic indexes tenacity (P) and strength (W) significantly decrease from 2.5% of damaged grains. From 5% there is a weakening of the protein fraction in Mixolab test.

7 – The pasta quality, considering the absorption of water, cooking loss, total organic matter (TOM) and sensory evaluation was not affected by the presence of wheat bugs damaged grains.

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ANEXOS

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Population density and distribution of wheat bugs infesting durum wheat in Sardinia, Italy

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Abstract

Wheat is a very important crop in Italy, and is infested by wheat bugs belonging to the genera *Eurygaster* (Hemiptera: Scutellaridae) and *Aelia* (Hemiptera: Pentatomidae). Many wheat bug infestations have been reported in the north, south, and center of Italy, both in the past as well as recently. The present study was carried out in Sardinia, Italy, during two years (2007 and 2008). The objective of this study was to determine the species and distribution of wheat bugs in durum wheat fields in Sardinia, and to estimate their population density in order to know the incidence of the pest on the island. Sampling took place twice a year (May and June) in three zones, representative of durum wheat cropping in the island. Four species of wheat bugs were found; the predominant species was *Eurygaster austriaca* (Schrank), followed by *Aelia germari* (Kuster), *Eurygaster maura* L., and *Aelia acuminata* L. The average density of wheat bugs was low (1.1 individuals/m^2), but in certain areas it was above the damage threshold (4 individuals/m^2). For this reason, the conclusion of the study is that this pest should be monitored in order to control outbreaks and prevent their further spread.

Sommario

Il frumento è una coltura molto importante in Italia e non è esente da infestazioni di cimici dei cereali appartenenti ai generi *Eurygaster* (Hemiptera: Scutellaridae) e *Aelia* (Hemiptera: Pentatomidae). Molte infestazioni di cimici dei cereali sono state segnalate nel Nord, Sud e Centro Italia, nel passato come di recente. Questo studio è stato condotto in Sardegna (Italia) nel corso di due anni (2007 e 2008). L'obiettivo di questo studio è determinare le specie e la distribuzione delle cimici dei cereali presenti nei campi di frumento duro in Sardegna, e stimare la loro densità di popolazione al fine di conoscere l'incidenza di questi insetti nocivi nell'isola. Il campionamento ha avuto luogo due volte l'anno (maggio e giugno) in tre zone, rappresentative della coltura del frumento duro dell'isola. Quattro specie di cimici dei cereali sono state trovate, la specie predominante era l'*Eurygaster austriaca* (Schrank), seguita da *Aelia germari* (Kuster), *Eurygaster maura* L. e *Aelia acuminata* L. La densità media delle cimici dei cereali è stata bassa (1,1 individui/m^2) , ma in alcune zone è stata al di sopra della soglia di danno (4 individui/m^2). Per questo motivo, la conclusione dello studio è che le cimici dei cereali devono essere monitorate per controllare e prevenire i focolai e la loro ulteriore diffusione.

Keywords: *Aelia*, density, *Eurygaster*, Hemiptera-Heteroptera, sunn pest
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Introduction

The names "sunn pest" and "wheat bug" refer to different species in the genera *Eurygaster* (Hemiptera: Scutellaridae) and *Aelia* (Hemiptera: Pentatomidae). Wheat bugs are widely distributed in various areas of Europe, Asia, and North Africa (Paulian and Popov 1980). An estimate of more than 15 million hectares of cereal, mainly wheat and barley, are infested annually in Syria, Iraq, Iran, Turkey, Afghanistan, and Lebanon, as well as in Central Asia and the Caucasus, Bulgaria, and Romania (El Bouhssini et al. 2002).

The genus *Eurygaster* includes 15 species (Froeschner 1988a; Javahery et al. 2000; Göllner-Scheiding 2006), of which only three are cereal pests, namely *Eurygaster integriceps* (Puton), *Eurygaster maura* L., and *Eurygaster austriaca* (Schrank) (Paulian and Popov 1980). *E. integriceps* is found from southern Europe up to China, although it is absent from the Iberian Peninsula; *E. maura* is present in Europe, North Africa, and central Asia; and finally, *E. austriaca* extends across the Euromediterranean region up to central Asia (Göllner-Scheiding 2006).

The genus *Aelia* includes 16 species (Froeschner 1988b; Derjanschi and Péricart 2005; Rider 2006), of which both *Aelia acuminata* L. and *Aelia rostrata* (Boheman) are known to be important cereal pests. In addition, *Aelia germari* (Kuster) and *Aelia klugii* (Hahn), among other *Aelia* species, can cause occasional damage (Paulian and Popov 1980). Both *A. acuminata* and *A. klugii* are Palaearctic species, and *A. rostrata* is present in the Euromediterranean region, extending eastwards up to India, whereas *A. germari* is found only in the Mediterranean basin (Rider 2006).

Wheat bug populations are generally univoltine, with the exception of certain *Aelia* species (Javahery 1996). In the spring, adults that have overwintered copulate and oviposit in the cereal fields, and the new generation of adult wheat bugs appears after going through five nymphal stages (Voegelé 1996). In autumn and winter, these adults undergo diapause after migrating over considerable distances or dispersing locally to overwintering sites (Brown 1965; Javahery 1996; Voegelé 1996). *Aelia* spp. and *Eurygaster* spp. undergo obligate diapause throughout their geographical range, and the diapause is influenced both by photoperiod and temperature (Javahery 1996). They hibernate as adults in various shelters including stones, dry leaves, and grass clumps (Voegelé 1996). All wheat bugs overwinter until temperatures rise in spring, at which time they move to cereal fields to feed and mate. The adults die soon after completing oviposition. Feeding in spring is essential prior to the first mating and oviposition for both sexes (Javahery 1996). Some *Eurygaster* and *Aelia* species are strongly migratory (> 20 km) while others are sedentary or only subject to very minor dispersion. Whether or not *Eurygaster* spp. and *Aelia* spp. invade areas that appear to be ecologically suitable may be explained according to wind direction (Brown 1965). Damage to the crop is proportional to the density of wheat bugs. Population density is directly related to hibernation success, which in turn depends on the accumulation of fat reserves prior to hibernation (Donkstoff 1996). Changes in population densities and outbreaks of these insects are largely determined by external abiotic and biotic factors. Climatic conditions, especially temperature and rainfall, play an important role in the population dynamics of wheat bugs. Continuous rainfall delays wheat bug activity, and long periods of high humidity in the overwintering sites cause mortality (Javahery 1996). Among the natural enemies observed, the most important belong to Hymenoptera, Diptera, and Fungi (Voegelé 1996) ,and they contribute to the regulation of wheat bug populations. Field margins are the main source of many natural enemies of this pest (Tshernyshev et al. 2010).

The economic importance of wheat bug damage is due to crop losses and/or quality loss of wheat (Kinaci and Kinaci 2004), semolina (Ozderen et al. 2008; Köksel et al. 2009; Salis et al. 2010), or flour (Hariri et al. 2000; Sivri et al. 1999, 2004; Aja et al. 2004; Vaccino et al. 2006; Werteker and Kramreither 2008). The feeding activity of wheat bugs also heavily affects the germination percentage of wheat (Bin et al. 2006). Both nymphs and adults of *Eurygaster* spp. and *Aelia* spp. cause a reduction of wheat quality when they insert their piercing-sucking mouthparts in the kernels and extract the substances within. In order to facilitate the suction of the nutritional elements of the endosperm, the kernels are digested externally by injecting saliva rich in proteases (Sivri et al. 1998; Konarev et al. 2011) and amylases (Kazzazi et al. 2005). The detrimental effect of such proteases on baking quality is very high, even when only 3–5% of kernels are damaged, and dramatically increases for values higher than 10% (Karababa and Ozan 1998; Hariri et al. 2000).

In Italy, wheat is not free of wheat bugs. Malenotti (1931) reported a heavy infestation of *A. acuminata* in 1931 in the province of Verona, and *E. maura* and *Eurygaster hottentota* F. were also found. In 1932–1933, a heavy infestation of *A. rostrata* was recorded in the provinces of Verona, Mantova, and Brescia, and *E. maura* was also found (Malenotti 1933). Less important infestations have been registered in the south of Italy, particularly in the Puglia region (Genduso and Di Martino 1974). Severe infestations of *A. rostrata*, together with the presence of *E. maura* and *E. austriaca*, were registered in Sicily in 1973–1975 (Genduso 1977). In 1998–1999, significant attacks of *E. maura*, and to a lesser extent *E. austriaca* and *Aelia* spp., were reported in Piedmont and on localities in the provinces of Alessandria and Asti (Tavella and Migliardi 2000). In 2000, *A. rostrata* was recorded in Sicily (Spina 2000). In 2005, a heavy infestation of *Eurygaster* spp. on soft wheat occurred in central Italy (Val di Chiana, Toscana) and required an insecticide treatment (Bin et al. 2006).

Durum wheat (*Triticum turgidum* L. var *durum*) is one of the most important crops in Italy, a country that generates around 50% of the total durum wheat production of the European Union, and around 15% of the world production (Sgroi and Fazio 2008). In 2008, Italy produced approximately 5.2 million tonnes of durum wheat (ISTAT 2010). Durum wheat constitutes ~70% of the total area cultivated with wheat in Italy. In Sardinia, durum wheat is the most widespread crop; it is grown on about 84,000 hectares, with an average production of about 134,000 tonnes per year (ISTAT 2010). Durum wheat is a very ancient crop in the Mediterranean basin and is used mainly to manufacture pasta, as well as for baking traditional types of bread (Quaglia 1988) with a particular interest both from an economic and a cultural point of view (Dexter and Marchylo 2000); the carasau bread, for example, made from durum wheat, is one of the most important products of the Sardinian bread making tradition (Dettori et al. 2002).

No studies on the distribution and density of wheat bugs have been carried out in Sardinia. Considering the importance of durum wheat in the Sardinian economy, a detailed

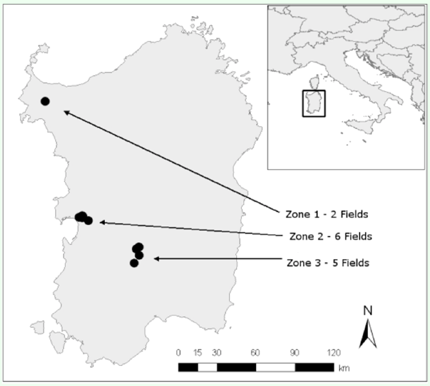

Figure 1. Zones and location of the fields sampled in Sardinia, Italy. High quality figures are available online.

knowledge of the species' distribution is required as a first step to develop sustainable management options for the improvement of the quality of wheat.

The aim of this study was to determine the species of wheat bugs present in the durum wheat fields of Sardinia, Italy, to explore their distribution in the island, and to estimate their population density in order to know the incidence of the pest on the island.

Material and Methods

The survey of wheat bugs was conducted during 2007 and 2008 in 13 durum wheat fields distributed in three different zones (Zone 1, Zone 2, and Zone 3), which were representative of durum wheat cropping in Sardinia (Figure 1; Table 1). Fields were selected at random within each zone. The number of sampled fields in each zone was proportional to the cultivated area. In the surveyed fields, neither pesticides nor fungicides were used, according to common agricultural practice in the region. In each field, insects were collected along six transects. Three transects covered the entire field edge, and the other three covered the interior of the field, following the protocol described by Pérez-Rodríguez et al. (2008). The field edge was considered to be the area between the border of field and two linear meters into the field, while the remaining part of the field was considered to be the interior. Along each transect, insects were collected in 15 regularly spaced sampling points. At each sampling point, an entomological sweep net with an opening of 0.17 m^2 was swept once over the cereal spikes in order to collect bugs. In other words, the total area sampled per transect was 2.55 m^2. Considering six transects per field, a total of 90 points were sampled, equivalent to 15.3 m^2 sampled per field. Sampling took place twice a year, at

Table 1. Geographic coordinates, altitude, and areas of sampled fields and meteorological stations of each zone.

Sample location		Geographical coordinates		Altitude	Area
Zone	Exact location	Latitude	Longitude	(m a.s.l.)	(ha)
1	Field 1	40° 43' 51.36" N	8° 19' 28.33" E	67	7
	Field 2	40° 43' 41.92" N	8° 19' 20.52" E	70	8
	Meteorological station (Olmedo-SS)	40° 39' 43" N	8° 21' 44" E	32	–
2	Field 3	39° 55' 26.74" N	8° 34' 54.93" E	3	8
	Field 4	39° 55' 27.12" N	8° 35' 7.14" E	2	5
	Field 5	39° 54' 24.58" N	8° 34' 38.43" E	4	1
	Field 6	39° 53' 29.98" N	8° 37' 40.60" E	8	1.1
	Field 7	39° 53' 33.13" N	8° 37' 43.56" E	7	3.3
	Field 8	39° 54' 8.22" N	8° 33' 10.32" E	1	13
	Meteorological station (Milis - OR)	40° 03' 58" N	8° 38' 42" E	125	–
3	Field 9	39° 38' 51.04" N	8° 58' 45.21" E	128	7.5
	Field 10	39° 42' 4.19" N	8° 58' 53.30" E	185	2.5
	Field 11	39° 42' 11.63" N	8° 58'57.01"E	190	2.3
	Field 12	39° 41' 50.40" N	8° 57' 2.34" E	181	1.4
	Field 13	39° 35' 31.97" N	8° 56' 50.11" E	95	5
	Meteorological station (Sardara - CA)	39° 36' 02" N	8° 51' 26" E	197	–

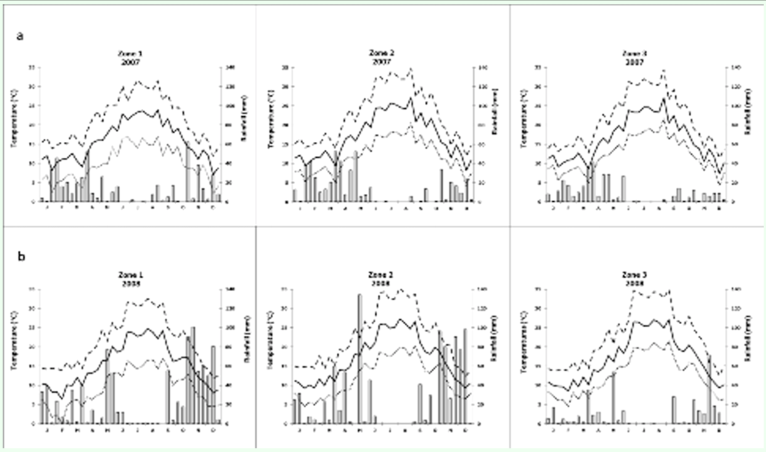

Figure 2. Rainfall (bars), average (solid line), maximum (dashed line), and minimum (dotted line) temperatures from 1 January to 31 December in 2007 (a) and 2008 (b). Rainfall values are sums, and temperature values are means, over 10-day periods. High quality figures are available online.

the beginning of grain filling (1–10 May) to account for the initial population of bugs, and at grain maturation stage (10–20 June), just before harvest, to account for the bugs' final population. The insects collected were kept separately according to transect. Insects were preserved with ethyl acetate to keep them in good condition until identification of species and development stage (adults and nymphs) in the laboratory. The species were identified under the binocular microscope, and genitalia were studied when necessary. Species were identified according to Vidal (1949), Stichel (1957), Kis (1984), Tamanini (1988), and Ruiz et al. (2003).

Meteorological data of each zone were taken from the nearest meteorological stations to the sampled fields (Table 1). Maximum and minimum temperature and rainfall were recorded daily. The study area is characterized by a typical Mediterranean climate with long, hot, dry summers and short, mild, rainy winters. The climatic variables measured for the three zones in 2007 and 2008 are reported in Figure 2. The rainfall for Zone 1 and Zone 2 was very similar within the same year, around 500 mm in 2007 and around 900 mm in 2008. Zone 3 was much drier both years, with a rainfall of 350 mm. In 2007, Zone 2 registered the highest mean temperature (17.1º C) with respect to Zone 3 (16.3º C) and Zone 1 (15.8º C). In 2008, Zone 2 and Zone 3 registered the same mean temperature (16.9º C), while Zone 1 was the coldest zone both years with a mean temperature of 15.5º C.

Differences (in individuals/m^2) between species, zones, sampling dates, development stage, and field zone were determined by fitting a generalized linear model to the data and estimating the dispersion parameter by maximum likelihood using the procedure GENMOD from the SAS software package (SAS Institute 2009) with log as a link function. Species, zone, sampling date, and field zone were considered to be fixed factors. Differences between means were computed, analyzing multiple pairwise differences with Tukey's test. Analyses for differences in in-

Table 2. Percentage of the different species of the genus *Eurygaster* and *Aelia* collected per year and zone. Values are expressed in percentage of the total number of adults captured during each year (n = 347 in 2007; n = 291 in 2008).

Genus	Species	2007				2008			
		Zone 1	Zone 2	Zone 3	Total	Zone 1	Zone 2	Zone 3	Total
Eurygaster	*E. austriaca*	0.6%	54.7%	18.4%	73.7%	1.7%	50.9%	24.1%	76.6%
	E. maura	1.4%	7.5%	0.0%	8.9%	0.3%	4.1%	0.3%	4.8%
Total *Eurygaster*		2.0%	62.2%	18.4%	82.6%	2.0%	55.0%	24.4%	81.4%
Aelia	*A. germari*	0.9%	9.5%	3.5%	13.9%	0.7%	9.3%	3.4%	13.4%
	A. acuminata	0.0%	3.5%	0.0%	3.5%	0.0%	4.5%	0.7%	5.2%
Total *Aelia*		0.9%	13.0%	3.5%	17.4%	0.7%	13.8%	4.1%	18.6%
Total Adults		**2.9%**	**75.2%**	**21.9%**	**100%**	**2.7%**	**68.7%**	**28.5%**	**100%**

sect densities between field zones (interior and edge) based on transect data showed no significance, and consequently, density data from all transects were pooled by field. Subsequent statistical analyses were performed using insect densities per field.

Results and Discussion

Genera and species of wheat bugs in Sardinia, and geographic distribution

During the two years of sampling in the 13 durum wheat fields of Sardinia, two genera, *Eurygaster* and *Aelia*, and four species, *E. austriaca*, *E. maura*, *A. germari*, and *A. acuminata*, were identified. Other species of *Eurygaster* and *Aelia* that have been cited in Sardinia, such as *E. hottentotta*, *A. rostrata*, *Aelia notata* (King), and *A. klugii*, were not found. Other species reported in other parts of Italy (Servadei 1952; Tamanini 1988; Faraci and Rizzotti Vlach 1995; Derjanschi and Péricart 2005; Fauna Europaea 2011), such as *E. integriceps*, *Eurygaster testudinaria* (Geoffroy), *Eurygaster dilaticollis* (Dohrn), and *Aelia sibirica* (Reuter), were not found either.

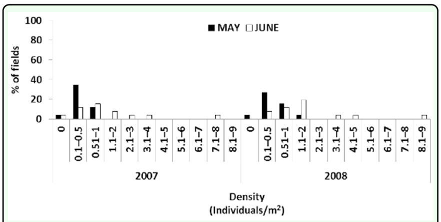

Figure 3. Frequency classes of wheat bug densities (individuals/m^2) found in fields sampled in May and June in 2007 and 2008. High quality figures are available online.

The results regarding the different species collected from each zone are shown in Table 2, in which data is expressed as percentage of total adults captured per year. The most abundant species was *E. austriaca*, which represented 75.1% of the total number of adults collected, followed by *A. germari* (13.6%), *E. maura* (7.1%), and *A. acuminata* (4.2%). These four species were present in all zones except for *A. acuminata*, which was never found in Zone 1. In all the zones and years, the most abundant species was *E. austriaca*, with an exception in Zone 1 in 2007, where *E. maura* was the most abundant species.

Density of wheat bugs in durum wheat fields

Frequency classes of wheat bug densities

In 2007 and 2008, about 80% and 65% respectively of the fields sampled registered a very low density of wheat bugs, between 0 and 1 individuals/m^2 (Figure 3). In June in both years, the density of wheat bugs was higher compared to May, and approximately half of the fields registered a density that ranged from 0.5 to 2 individuals/m^2 (Figure 3). There were very few fields with densities above 4 individuals/m^2, established as the damage threshold by Paulian and Popov (1980). The exceptions were observed in Zone 2, which had 4.3 and 7.3 individuals/m^2 in field 3 in June 2007 and 2008 respectively, and 8.2 individuals/m^2 in field 8 in June 2008.

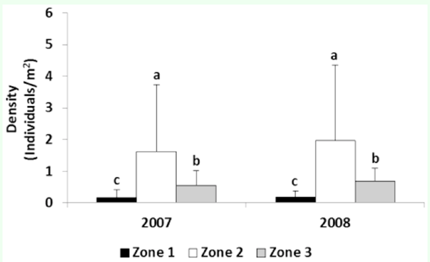

Figure 4. Mean densities of wheat bugs per year in the different zones. In each year, the same letter indicates no significant differences ($p < 0.05$). Bars represent standard deviation (n = 312). High quality figures are available online.

Total density of wheat bugs per year and zone

A total of 867 wheat bugs (638 adults and 229 nymphs) were collected. The average density of wheat bugs sampled in Sardinia in 2007 (0.98 individuals/m^2) was not significantly different ($p > 0.05$) than 2008 (1.19 individuals/m^2). Population densities, however, varied significantly between zones (Figure 4). The average density in Zone 2 was significantly higher than Zone 3 and Zone 1 (Figure 4). Many factors influence the optimal conditions for the development of wheat bugs, such as climatic conditions, areas cropped, parasites, and overwintering sites (Javahery 1996; Popov et al. 2003; Kutuk et al. 2010), and they can determine different population densities. During the period 20 April – 20 June, immediately before the first sampling (1–10 May) until the second sampling (10–20 June), the average temperature in Zone 2 was the highest (in 2007: Zone 2, 19.3º C; Zone 3, 18.7º C; Zone 1, 18.5º C; in 2008: Zone 2, 18.7º C; Zone 3, 18º C; Zone 1, 17.5º C). In both years, during the period considered above, the average maximum temperature was highest in Zone 2, whereas Zone 1 always presented the lowest average minimum temperature. Higher temperatures stimulate the development of wheat bugs (Javahery 1996; Iranipour et al. 2010) and could explain the different densities in the three zones (Figure 4).

The most abundant genus, statistically significant in both years of the study, was *Eurygaster*, with 0.80 individuals/m^2 in 2007 and 0.83 individuals/m^2 in 2008, while *Aelia* had a density of 0.18 individuals/m^2 and 0.37 individuals/m^2 in 2007 and 2008 respectively ($p < 0.05$).

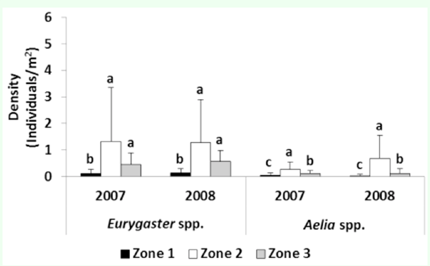

Figure 5. Mean densities of *Eurygaster* spp. and *Aelia* spp. per year and zone (B). Within each genus and year, the same letter indicates no significant differences ($p < 0.05$). Bars represent standard deviation (n = 312). High quality figures are available online.

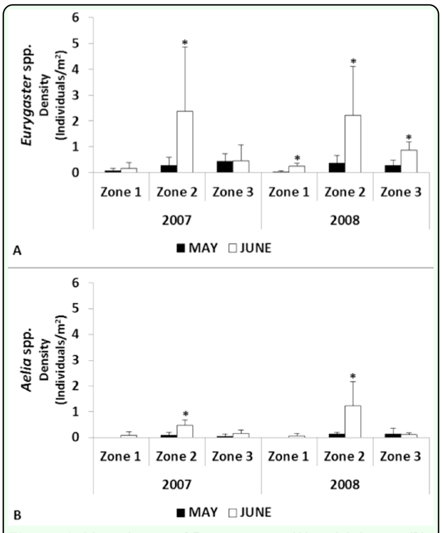

Figure 6. Mean density of *Eurygaster* spp. (A) and *Aelia* spp. (B) per year in the different zones in May and June. Data followed by an asterisk (*) indicates significant differences ($p < 0.05$) within each zone in each year. Bars represent standard deviation (n = 312 for each genus). High quality figures are available online.

The population density of each genus (Figure 5) showed similar results as total density. In both years, the density of the genus *Eurygaster* was significantly higher in Zone 2 and Zone 3 compared to Zone 1. Similarly, the density of the genus *Aelia* was always significantly higher in Zone 2 than in Zone 3 and Zone 1. The density of *Aelia* was higher in Zone 3 than in Zone 1 in 2007 and 2008 (Figure 5).

Density of wheat bugs by sampling date (May–June) and by development stage (nymphs and adults)

Densities of wheat bugs were statistically lower ($p < 0.05$) in the first sampling date (May) than in the second sampling date (June) in Zone 1 (in 2007, 0.07 individuals/m^2 and 0.26 individuals/m^2 respectively; in 2008, 0.03 individuals/m^2 and 0.33 individuals/m^2 respectively) and Zone 2 (in 2007, 0.38 individuals/m^2 and 2.84 individuals/m^2 respectively; in 2008, 0.49 individuals/m^2 and 3.45 individuals/m^2 respectively). According to the wheat bugs' life-cycle, they overwinter in or under diverse shelters (stones, dry leaves, grass clumps) until they move to cereal fields to feed and mate. Few adults were found in wheat fields in May (< 1 individuals/m^2). No nymphs were found in May, as reproductive activity had not yet begun. It is important to know the density of the overwintered adults in wheat fields because it is associated to nymphs and new-adults generation (Kutuk et al. 2010), which is the most detrimental to wheat crop.

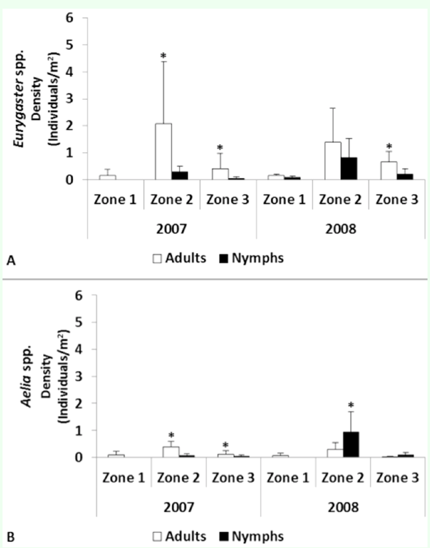

Figure 7. Mean densities of adults and nymphs of *Eurygaster* spp. (A) and *Aelia* spp. (B) in June. Data followed by an asterisk (*) indicates significant differences ($p < 0.05$) within each zone in each year. Bars represent standard deviation (n = 312 for each genus). High quality figures are available online.

In June, when wheat was at the end of grain-filling and maturation, overwintered adults had already reproduced and a mixed population of nymphs and adults was found, resulting in a higher population density with respect to May, as explained above. These findings, regarding wheat bugs densities in May and June, agree with other studies, such as Hariri et al. (2000) in Syria, Popov et al. (2003) in Romania, and Kutuk et al. (2010) and Canhilal et al. (2005) in Turkey.

The genus *Eurygaster* showed significantly higher densities ($p < 0.05$) in June than in May only in Zone 2 in 2007 and in all the zones in 2008 (Figure 6A). The densities of the genus *Aelia* were significantly higher in Zone 2 in June of both years (Figure 6B).

As regards the density of adults and nymphs in June, significantly more *Eurygaster* spp. adults than nymphs were recorded in Zone 2 in 2007 and in Zone 3 both years (Figure 7A). For *Aelia* spp., significantly more adults than nymphs were found in Zone 2 and Zone 3 in 2007 (Figure 7-B). The only exception is for

Aelia spp. in Zone 2 in 2008, when significantly more nymphs than adults were found (Figure 7B). Finally, in some cases no nymphs were recorded from the genus *Eurygaster* in Zone 1 in 2007 and from the genus *Aelia* in Zone 1 for both years (Figure 7AB).

Density by field zone: edge or interior

No significant differences in density between the edge and the interior of the field were found for either *Eurygaster* spp. or *Aelia* spp., neither in any of the years studied nor in the two sampling periods, except for *Aelia* spp. in June 2007 in Zone 2, in which its density was significantly higher ($p < 0.05$) in the edge of the field (0.63 individuals/m^2) than in the interior (0.31 individuals/m^2). This result was possibly due to the fact that many sampled fields were side by side in a continuum without a properly limiting edge. Conversely, in other studies (Afonina et al. 2001; Pérez-Rodriguez et al. 2008) in which the wheat fields were sampled in the edge and in the interior, differences were found between the field zones. In a region of South Russia, for example, *E. integriceps* began to colonize the field from its edges; however, the following generations were more abundant in the center of the field (Afonina et al. 2001). In northeast Spain, differences between density in the edge and the interior of the field were found only for *Aelia* spp., but not for *Eurygaster* spp. (Pérez-Rodriguez et al. 2008). It is important to know the distribution of the wheat bugs in fields in order to select the appropriate sampling method to be used. This could, moreover, permit the early detection of the wheat bugs before copulation and oviposition, which is very important for the control of this pest.

Conclusions

The predominant species of wheat bug found in the durum wheat fields sampled in Sardinia was *E. austriaca*, followed by *A. germari*, *E. maura*, and *A. acuminata*. Bug density varied significantly according to the zone, being much higher in Zone 2 than in Zone 1 and Zone 3. The average density of bugs was low (1.1 individuals/m^2), but in certain areas it was above the damage threshold (4 individuals/m^2). Therefore, it would be necessary to monitor the wheat bugs in order to detect outbreaks before they produce economic damage and spread to other areas. The overall density of wheat bugs was lower in May than in June. No nymphs were found in May, only adults. No significant differences were found in the distribution of bugs between field edge and interior, except for *Aelia* spp. in Zone 2 in 2007.

Considering how important durum wheat crops are to the Italian economy, and in view of the infestations of wheat bugs in several Italian regions, it would be desirable to carry out more studies on wheat bugs in the durum wheat production areas. A better understanding of the spatial-temporal population trends is needed in order to develop and apply a cost-effective and environmentally sound pest management program for the control of these pests.

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Prolamin proteins alteration in durum wheat by species of the genus *Eurygaster* and *Aelia* (Insecta, Hemiptera)**

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Abstract

Wheat bugs are widely distributed in various areas of Europe, Asia and North Africa. Species belonging to the genus *Eurygaster* and *Aelia* pierce wheat kernels affecting protein quality. The effects of these insects' feeding activity have been studied mainly in bread wheat (*Triticum aestivum* L.). This study provides information on the degradation of prolamin proteins (glutenins and gliadins) of bug-damaged durum wheat (*Triticum turgidum* L. var *durum*) in six cultivars grown in Sardinia (Italy). Samples of whole flour mixture of 70% sound wheat and 30% damaged wheat were hydrated and incubated at two temperatures (45 and 4°C), for different periods of time (0, 1 and 3 h). Glutenin and gliadin content was analysed using free zone capillary electrophoresis. The presence of bug-damaged kernels had influence on the quality of durum wheat proteins. Glutenins were rapidly degraded independently to incubation temperature. Gliadin degradation, however, took place with dependence on temperature and incubation time. Therefore glutenin degradation was possibly not due solely to the activity of proteolytic enzymes but also to some other as yet unknown factor linked to wheat bugs' feeding activity.

Additional key words: gliadin, glutenin, protein quality, *Triticum turgidum* L. var *durum,* wheat bugs.

Resumen

Alteración de las prolaminas en trigo duro por especies del género *Eurygaster* y *Aelia* (Insecta, Hemiptera)

Los chinches de los cereales tienen una amplia distribución en diversas áreas de Europa, Asia y Norte de África. Especies pertenecientes a los géneros *Eurygaster* y *Aelia* se alimentan picando los granos de trigo afectando a la calidad de las proteínas. Esta alteración se ha estudiado principalmente en trigo blando (*Triticum aestivum* L.). El presente estudio aporta información sobre la degradación de las prolaminas (gluteninas y gliadinas) del trigo duro (*Triticum turgidum* L. var *durum*) dañado por los chinches de los cereales en seis variedades cultivadas en Cerdeña (Italia). Muestras de harina integral, con un 70% de trigo sano y un 30% de trigo dañado se incubaron a dos temperaturas (45 y 4°C), y a diferentes tiempos (0, 1 y 3 h). El contenido de las gluteninas y gliadinas se analizó mediante electroforesis capilar zonal. La presencia de granos picados por los chinches de los cereales influyó en la calidad de las proteínas del trigo duro. Las gluteninas se degradaron rápidamente, con independencia de la temperatura de incubación. En cambio, la degradación de las gliadinas resultó ser dependiente de la temperatura y del tiempo. Ante estos resultados, cabe plantearse la posibilidad de que la degradación de las gluteninas no sea debida sólo a la actividad de las proteasas sino también a algún otro factor ligado a la actividad alimenticia de los chinches de los cereales, todavía por determinar.

Palabras clave adicionales: calidad de las proteínas, chinches de los cereales, gliadinas, gluteninas, *Triticum turgidum* L. var *durum*.

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Abbreviations used: A-PAGE (acid polyacrylamide gel electrophoresis), DTT (dithiothreitol), FZCE (free zone capillary electrophoresis), HMW-GS (high molecular weight glutenin subunits), IDA (iminodiacetic acid), LMW-GS (low molecular weight glutenin subunits), RP-HPLC (reverse phase-high performance liquid chromatography).

Introduction

Wheat (*Triticum* spp.) is one of the three most important crops in relation to cultivated area (approximately 215 million hectares) and total global production (approximately 600 million tonnes) (FAOSTAT, 2008). The unique properties of wheat flour and dough enable the production of a wide range of products such as various types of bread, pasta, cakes and biscuits (Shewry *et al*., 1997).

Durum wheat (*Triticum turgidum* L. var *durum*) is a very important crop in Italy, the production in 2007 was approximately 4.1 million tonnes (ISTAT, 2008), around 15% of the world production. Durum wheat is used mainly for the manufacture of pasta as well as for baking traditional types of bread (Quaglia, 1988). The semolina obtained from durum wheat is the preferred prime matter for the manufacture of superior quality pasta (Feillet and Dexter, 1996).

Different factors can be detrimental to wheat crops, agronomical factors, environmental conditions, pests, diseases, etc., which can result in loss of quantity and quality of wheat, semolina or flour. Wheat bugs of the genus *Eurygaster* (Hemiptera, Heteroptera, Fam. Scutelleridae) and *Aelia* (Hemiptera, Heteroptera, Fam. Pentatomidae), also known as Sunn pest, affect both aforementioned aspects.

Eurygaster and *Aelia* wheat bugs are widely distributed in various areas of Europe, Asia and North Africa (Paulian and Popov, 1980) and an estimate of more than 15 million hectares of cereals (mainly wheat and barley) are infested annually in Syria, Iraq, Iran, Turkey, Afghanistan and Lebanon, as well as in Central Asia and the Caucasus, Bulgaria and Romania (El Bouhssini *et al*., 2002). There are long term studies focussed on integrated pest control (El Bouhssini *et al*., 2002; Parker *et al*., 2003) and on the effects on protein alteration caused by the salivary residues left in the kernels by the insects' feeding (Kretovich, 1944; Karababa and Ozan, 1998; Sivri *et al*., 1998, 1999, 2004; Hariri *et al*., 2000; Aja *et al*., 2004; Caballero, 2005; Ozderen *et al*., 2008; Werteker and Kramreither, 2008). Effects on the alteration of starch granules have been reported for *Nysius* spp. (Hemiptera, Heteroptera, Fam. Lygaeidae), a wheat bug present in Australia and New Zealand (Every *et al*., 1990; Lorenz and Meredith, 1998) but amylase activity is not involved in the wheat damage caused by *Aelia* spp. and *Eurygaster* spp. (Rosell *et al*., 2002a).

Both nymph and adult *Eurygaster* and *Aelia* wheat bugs insert their piercing-sucking mouthparts in the wheat kernels and extract the substances within. In order to facilitate the suction of the nutritional elements of the endosperm, the kernels are digested externally by injecting saliva rich in proteolytic enzymes (Sivri *et al*., 1998) and amylases (Kazzazi *et al*., 2005). Pierced kernels in the field usually continue to mature. When ripe, bug-damaged kernels present a whitish opaque spot and sometimes also a small black dot where the kernel was pierced (Hariri *et al*., 2000).

There is a general agreement that durum wheat protein content is the primary factor influencing rheological properties and pasta quality (D'Egidio *et al*., 1990; Novaro *et al*., 1993; Feillet and Dexter, 1996). Protein content and amino acid composition of wheat vary depending mainly on genotype and agro-climatic conditions (López-Bellido *et al*., 1998; Rharrabti *et al*., 2003; Dupont *et al*., 2006).

Gluten proteins are to a considerable extent responsible for the functional properties of flour. Wheat gluten consists of more than 50 protein components (Shewry *et al*., 1987) that have been traditionally classified into two groups, gliadins and glutenins (Wieser, 2007). Gliadins are soluble in aqueous alcohols (60-70% ethanol, 50% 1-propanol) and are present as monomeric proteins that lack inter-chain disulphide bonds, presenting instead intra-chain disulphide bonds (Shewry *et al*., 1997). Glutenins are insoluble in aqueous alcohols and consist of protein subunits present in polymers stabilised by inter-chain disulphide bonds. A reduction of these bonds results in subunits which are soluble in alcohol/water mixtures. Rheological properties of dough depend on both protein fractions (Shewry *et al*., 1997).

Although the effects of wheat bugs' feeding activity on bread wheat have been largely studied (Karababa and Ozan, 1998; Sivri *et al*., 1999, 2004; Hariri *et al*., 2000; Aja *et al*., 2004; Vaccino *et al*., 2006; Werteker and Kramreither, 2008) there are not many works regarding durum wheat and other species of the genus *Triticum*. There are various studies on the percentage of bug-damaged kernels necessary in order to seriously affect the kernel, flour, dough or bread quality parameters. Wheat samples which had more than 5% bug-damaged kernels changed their physicochemical properties and showed significantly lower quality (Karababa and Ozan, 1998; Hariri *et al*., 2000). The aim of this study was to investigate how the feeding activity of cereal bugs of the genus *Eurygaster* and/or *Aelia* affects glutenins and gliadins in durum wheat at different incubation conditions concerning temperature (45 and 4°C) and time (0, 1 and 3 h).

Material and methods

Wheat samples

Six cultivars (Karalis, Asdrubal, Claudio, Rusticano, Colosseo and Canyon) of durum wheat grown in extensive commercial fields near Villamar in the south-centre of Sardinia island (Italy) were tested. Those samples were provided by Laore, the Regional Agency of Sardinia for the Development of Agriculture. The samples showed a percentage of damaged kernels ranging from 1.6 to 4.1%. In each sample, bug-damaged kernels, characterized by a typical whitish opaque spot and also very often a black dot, were separated visually from sound kernels. Two series of sub-samples were prepared for each cultivar in order to carry out the protein analysis. One of the sub-samples consisted of only sound kernels without visible defects; the other sub-samples consisted of medium level bug-damaged kernels —*i.e.,* with a damage between 1/3 and 2/3 of the total grain surface—, lacking other visible defects. The sound kernels were ground using a Perten 3100 laboratory grinder to obtain wholemeal flour, whereas a Culloti laboratory grinder was used for the damaged kernels because of their scarcity. Both mills had a 0.8 mm sieve.

Protein alteration test, capillary electrophoresis analysis

For each cultivar, two types of samples of whole flour were worked out: on one hand, samples of sound flour (named «sound wheat», used to perform a control or stability test), and on the other hand samples of a blend of 70% sound flour and 30% bug-damaged flour (named «damaged wheat» used to perform a degradation test).

All samples, after hydration with deionised water, were tested using different incubation times (0 h = unincubated, 1 h = 1 hour and 3 h = 3 hours) and temperatures (45 and 4°C). Temperatures tested by authors in studies on bread wheat usually range from 37°C to 45°C (Sivri *et al*., 1998, 1999; Aja *et al*., 2004), whereas 4°C is a temperature that has not been tested previously.

The proportions of the blend of wheat, 70% sound wheat and 30% damaged wheat, as well as incubation conditions at 45°C were selected in order to provide a sufficient degree of protein degradation and also because they had already been tested on bread wheat (Sivri *et al*., 1999) therefore providing a comparative frame to our results. Incubation at 4°C was chosen to assess if degradation can be blocked. All the assays were carried out at least in duplicate.

A sequential protein extraction (albumins, globulins, gliadins and glutenins) was carried out on each sample following the protocol described by Bean and Lookhart (1998). For each cultivar, a sample of 100 mg of sound wheat or damaged wheat was hydrated with 1 mL of deionised water for incubation or to start off protein fractioning, specifically albumins. After stirring for 5 min the sample was centrifuged at 14,000 rpm for 5 min; the supernatant was discarded and the pellet resuspended using 1 mL of extraction buffer (50 mM Tris HCl, 50 mM KCl, 5 mM EDTA, at pH 7.8) so as to discard globulins; this operation was repeated twice, always stirring for 5 min and centrifuging at 14,000 rpm. The pellet was then resuspended using 1 mL of 50% 1-propanol (v/v) in order to extract the gliadins. The pellet was cleaned yet again with 1 mL of 50% 1-propanol. Finally, the glutenins were obtained by resuspending and stirring the pellet with 50% 1-propanol + 1% DTT (v/v) for 30 min and then centrifuging at 14,000 rpm for 5 min. For the samples incubated at 4°C, this temperature was maintained up to albumin and globulin extraction to avoid possible activity of the proteases on the gliadin and glutenin fraction. Gliadin and glutenin extracts were filtered at 45 µm.

Analysis of the different gliadin and glutenin subunits was carried out using FZCE. The process followed was similar to the method described by Bean and Lookhart (2000), but adjusted to a Hewlett-Packard CE and using a silex capillary tube (34 cm Polymicro Phoenix AZ, 25.5 cm $L_{D,}$ 50 µm i.d.) 50 mba × 8 s for injection of the samples, an IDA buffer (50 mM IDA, 20% acetonitrile and 0.05% hydroxypropylmethylcellulose) at 30 kV and 45°C.

Gliadin and glutenin alteration was assessed by comparing the electropherograms obtained from the sound wheat and damaged wheat in the incubation conditions described. The alterations in the total area (total quantity of gliadins or glutenins expressed in mAU*s) of the electropherograms were considered to be signs of protein alteration.

Statistical analysis

The results express the mean values obtained from two repeated trials. Factorial analysis of variance of glutenins and gliadins was carried out using the factors cultivar, temperature and incubation time. The results were analyzed using the Tukey method. The statistical analysis of data was carried out using the SAS software. A level of significance of 0.05 ($P < 0.05$) was used throughout the study.

Results

Glutenins

The stability test proved that glutenins from sound wheat were stable, with no significant differences in the amount of glutenins between the sound wheat unincubated and the sound wheat incubated for 3 h at 45°C. This allowed to use the mean value of duplicated sample of unincubated sound wheat as a reference value in order to evaluate degradation; an example of this can be observed in the stability test of cv. Karalis, shown by the equivalent electropherogram area in Figure 1a-1b. The degradation test proved (Fig. 1c-1d) that the glutenins of the damaged wheat, on the other hand, were degraded rapidly even in unincubated samples (Table 1, Fig. 2), with an average loss compared to the sound wheat samples of –65%. These differences were significant in all wheat cultivars when compared to the sound wheat (Fig. 2). Degradation was slightly higher when the damaged wheat samples were incubated (Table 1). The average degradation for 1 h was –79% at 4°C and –78% at 45°C. The average degradation for 3 h at both 4°C and at 45°C was –86% (Table 1). Differences between sound wheat samples and incubated (1 h and 3 h) damaged wheat samples were significant for all cultivars (Fig. 2). Not all samples showed significant differences between incubation at 1 h or 3 h at the same temperature (Fig. 2). For each cultivar, there were no significant differences between incubation at 45°C and at 4°C for the same incubation time, with the exception of Rusticano and Asdrubal cultivars incubated for 1 h and 3 h respectively (Table 1). Thus it can be stated, with some exceptions, that glutenins were degraded to the same degree at 45°C as at 4°C.

Gliadins

The stability test proved that gliadins from sound wheat were stable, with no significant differences in the amount of gliadins between the sound wheat

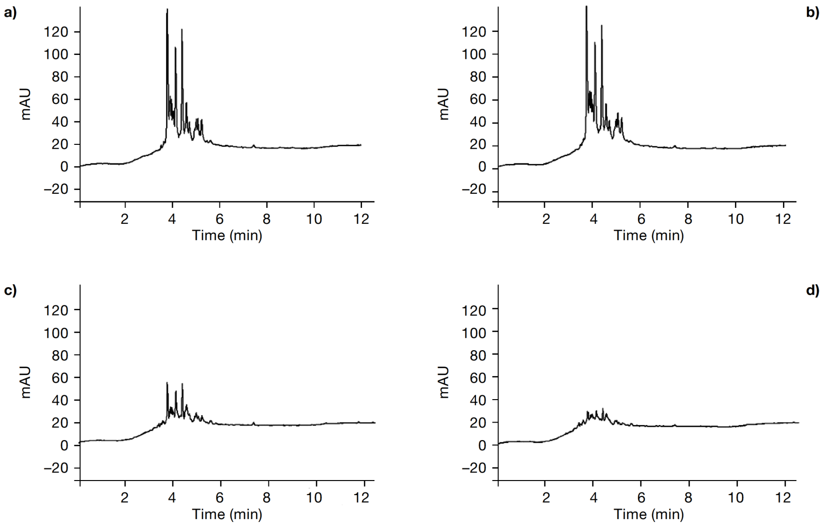

Figure 1. FZCE electropherograms of cv. Karalis glutenins (50% 1-propanol + 1% DTT) from sound wheat unincubated (a) and incubated at 45°C 3 h (b), and damaged wheat incubated at 45°C for 1 h (c) and 3 h (d).

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Table 1. Quantification of the alteration of total glutenins and gliadins of the damaged wheat at different temperatures (45°C, 4°C) and different incubation times (0 h, 1 h and 3 h) compared to sound wheat, expressed as loss (–) or gain (+) of area, in percentage and mAU*s (total area). For each cultivar, data within columns followed by the same letter are not significantly different ($P < 0.05$).

	Karalis		Asdrubal		Claudio		Rusticano		Colosseo		Canyon		Mean*
	%	mAU*s	%	mAU*s	%	mAU*s	%	mAU*s	%	mAU*s	%	mAU*s	%
Total glutenins													
Sound wheat		3,718^{a}		4,250^{a}		3,822^{a}		3,950^{a}		3,823^{a}		4,418^{a}	
Damaged wheat 0 h 4°C	–63	–2,345^{b}	–60	–2,554^{b}	–65	–2,472^{b}	–58	–2,342^{b}	–73	–2,795^{b}	–69	–3,047^{b}	–65 ± 5.5
Damaged wheat 1 h 45°C	–75	–2,779^{c}	–73	–3,084^{c}	–82	–3,143^{c}	–66	–2,611^{c}	–84	–3,198^{c}	–87	–3,853^{cd}	–78 ± 8.0
Damaged wheat 1 h 4°C	–79	–2,920^{cd}	–70	–2,992^{c}	–85	–3,234^{cd}	–74	–2,926^{d}	–84	–3,224^{c}	–84	–3,704^{d}	–79 ± 6.0
Damaged wheat 3 h 45°C	–80	–2,987^{cd}	–86	–3,641^{d}	–88	–3,346^{cd}	–80	–3,157^{de}	–91	–3,489^{d}	–91	–4,009^{c}	–86 ± 4.9
Damaged wheat 3 h 4°C	–82	–3,053^{d}	–81	–3,454^{e}	–90	–3,451^{d}	–83	–3,271^{e}	–91	–3,492^{d}	–91	–4,008^{c}	–86 ± 4.8
Total gliadins													
Sound wheat		2,958^{ab}		3,511^{a}		3,179^{a}		3,242^{ab}		3,149^{ac}		3,862^{a}	
Damaged wheat 0 h 4°C	+4	+114^{a}	–11	–386^{ac}	+5	+175^{a}	+5	+167^{a}	+30	+943^{b}	–9	–330^{a}	+4 ± 14.6
Damaged wheat 1 h 45°C	+11	+334^{a}	–18	–637^{abc}	–32	–1,023^{b}	–22	–718^{b}	–18	–559^{a}	–38	–1,485^{b}	–20 ± 17.2
Damaged wheat 1 h 4°C	–9	–264^{ab}	–16	–557^{abc}	–6	–179^{a}	+8	+250^{a}	+17	+551^{bc}	–18	–689^{ac}	–4 ± 13.8
Damaged wheat 3 h 45°C	–56	–1,652^{c}	–41	–1,438^{b}	–55	–1,763^{c}	–50	–1,624^{c}	–70	–2,193^{d}	–66	–2,563^{d}	–56 ± 10.5
Damaged wheat 3 h 4°C	–24	–718^{b}	–31	–1,098^{c}	–6	–205^{a}	–5	–160^{ab}	–11	–332^{a}	–35	–1,342^{bc}	–19 ± 13.1

* Mean: mean of the alteration of the six cultivars ± standard deviation.

unincubated and the sound wheat incubated for 3 h at 45°C. This allowed to use the mean value of the duplicated sample of unincubated sound wheat as a reference value in order to evaluate degradation; an example of this can be observed in the stability test of cv. Karalis shown by the equivalent electropherogram area in Figure 3a and 3b. The behaviour of gliadins in the damaged wheat samples differed strongly from that of glutenins. It was found that, in contrast to that of glutenins, degradation of gliadins was much lower and was time and temperature dependent (Table 1, Figs. 3c-3d and 4). Most of the unincubated (0 h) damaged wheat samples showed a total gliadin area increase (Table 1, Fig. 4), presumably due to the depolymerised glutenins. These increments ranged from +4% to +30% compared to the sound flour, with the exception of the cvs. Asdrubal and Canyon that lost –11% and –9%, respectively (Table 1). These differences, however, compared to the sound wheat, were only significant for the Colosseo cultivar (Fig. 4). For the damaged wheat samples incubated for 1 h at 45°C degradation ranged from –18% to –38% with the exception of the cv. Karalis where an increase of +11% was registered (Table 1). The differences were only significant, compared to the sound wheat, in the cvs. Claudio and Canyon (Fig. 4). There were minor variations in the damaged wheat samples incubated for 1 h at 4°C (from +17% to –18%, Table 1) and no significant differences when compared to the sound wheat (Fig. 4). However increases in gliadin degradation were significant for all cultivars,

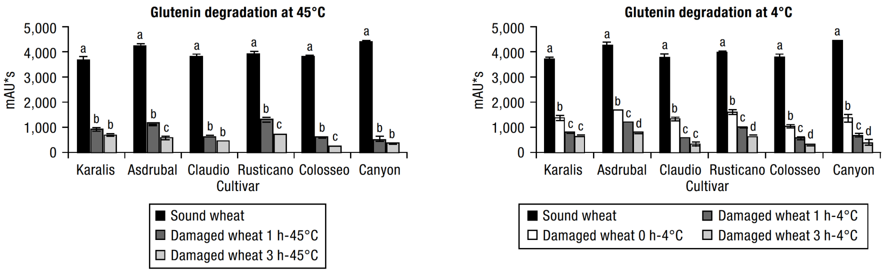

Figure 2. Results for glutenin degradation, comparing sound wheat and damaged wheat, in six durum wheat cultivars, at different temperatures (45 and 4°C) and different incubation times (0 h, 1 h and 3 h) expressed as total area in mAU*s. Bars describe standard deviation. For each cultivar at 45°C or at 4°C, the same letter indicates not significant differences (Tukey test, $P < 0.05$).

compared to the sound wheat, when damaged wheat samples were incubated for 3 h at 45°C (Fig. 4) In this case the average degradation percentage was –56% of the total gliadins (Table 1). Degradation of gliadins incubated for 3 h at 4°C, with an average degradation of –19%, was much lower than in samples incubated for 3 h at 45°C (Table 1). For each cultivar, there were significant differences between incubation at 45°C and at 4°C for 3 h. Above all, it can be stated that gliadins were not degraded to the same degree at 45°C as at 4°C.

Discussion

The previous results show that wheat bugs have a strong influence on durum wheat proteins determining quality loss. Total glutenin and gliadin alteration, due to the presence of bug-damaged kernels, was quantified and results were similar to those described in bread wheat (analysis perfomed only at high temperature). Glutenin results were very much alike to those obtained in bread wheat (Sivri *et al.*, 1998, 1999;

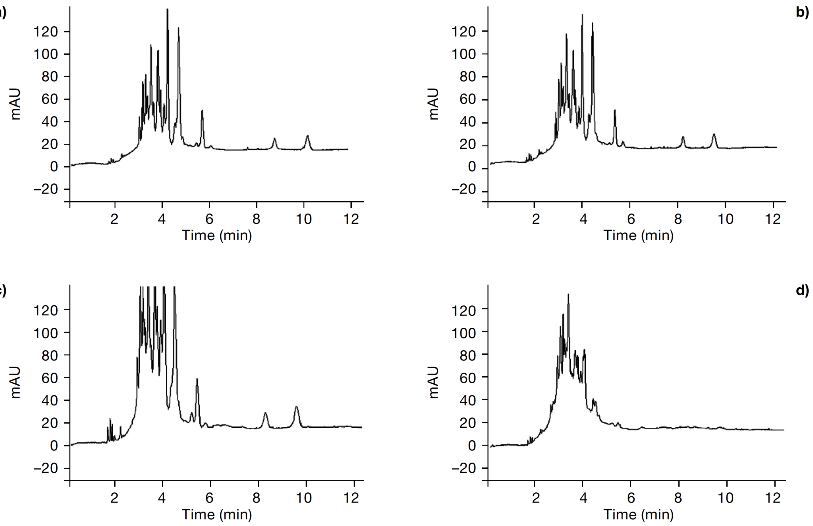

Figure 3. FZCE electropherograms of cv. Karalis gliadins (50% 1-propanol + 1% DTT) from sound wheat unincubated (a) and incubated at 45°C for 3 h (b), and damaged wheat incubated at 45°C for 1 h (c) and 3 h (d).

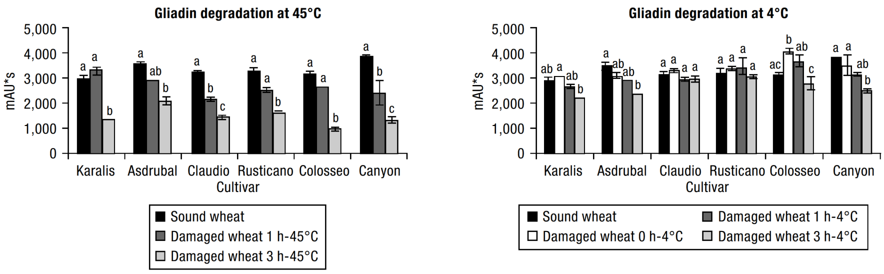

Figure 4. Results for gliadin degradation, comparing sound wheat and damaged wheat, in six durum wheat cultivars, at different temperatures (45 and 4°C) and different incubation times (0 h, 1 h and 3 h) expressed as total area in mAU*s. Bars describe standard deviation. For each cultivars at 45°C or 4°C, the same letter indicates not significant differences (Tukey test $P < 0.05$).

Rosell *et al.*, 2002b). In wheat cultivated in Turkey and manually infested by *E. maura* (L.) bugs, it was seen that after 30 min incubation, more than 80% glutenin was degraded, and increase of degradation was very low 1 h and 2 h after incubation (Sivri *et al.*, 1999). In that research, the proportions (2:1) of sound and damaged wheat in the blend were similar to those used in the present study, temperature of incubation was 37°C, and determination of reduced glutenins was done by (RP-HPLC). In bug-damaged bread wheat cultivated in Spain, a decrease of the glutenin fractions HMW-GS and LMW-GS determined with FZCE was also reported (Rosell *et al.*, 2002b). Regarding bug-damaged wheat, less information is available on gliadins than on glutenins. The initial increase in the area of gliadins observed in this study, probably due to the presence of glutenin degradation products with a higher electrophoretic mobility, was also described in bug-damaged bread wheat, as in the case of Spanish bread wheat samples and using FZCE (Rosell *et al.*, 2002b). In gliadins from bread wheat from Turkey incubated at 37°C, and analysed by A-PAGE, a decrease was also stated, concerning some new bands as well as the original gliadin bands; as in the case of durum wheat, the changes were more obvious with increasing incubation times and most of the gliadin bands were lost after 120 or 240 min of incubation (Sivri *et al.*, 1998).

In bug-damaged bread wheat a marked increase of free thiol groups during initial incubation was described (Pérez *et al.*, 2005). Results in durum wheat damaged by wheat bugs indicate that the alteration of the gliadins and glutenins initially begins with a fast depolymerisation of glutenins, independently of temperature, presumably due to a reduction of inter-chain disulphide bonds. The alteration continues, depending on the temperature, showing a general and specific degradation of gliadins and solubilised glutenins. Therefore, degradation of glutenins was possibly due not only to the action of protease enzymes, but also to another not yet determined salivary agent(s), that would act as a reductor. These durum wheat results could explain the modification of pasta (spaghetti) quality characteristics, referring to a loss of gluten quality, cooking values and a deterioration of sensory properties, when semolina from bug-damaged wheat is used to make pasta (Ozderen *et al.*, 2008; Köksel *et al.*, 2009). Similar consequences could be expected when baking certain traditional breads with flour obtained from damaged durum wheat, although no test has yet been performed to assess this hypothesis.

The final conclusion is that wheat bugs have a strong influence on durum wheat proteins that determines a quality loss. When hydrating durum wheat flour or semolina obtained from kernels damaged by wheat bugs the glutenins depolymerised quickly and almost in their totality. This degradation is independent of temperature. By contrast, the gliadins and the solubilised glutenins are degraded with less intensity and the degradation is time and temperature dependent.

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