Insecticidal Proteins from Mushrooms for controlling the Colorado Potato Beetle

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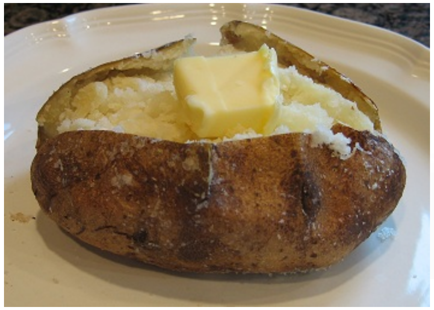
Department of Biotechnology, **Jožef Stefan Institute**, Ljubljana, Slovenia

Department of Biotechnology and Systems Biology, **National Institute of Biology**, Ljubljana, Slovenia

Plant health for sustainable agriculture, Ljubljana, 11th – 12th May 2015

POTATO



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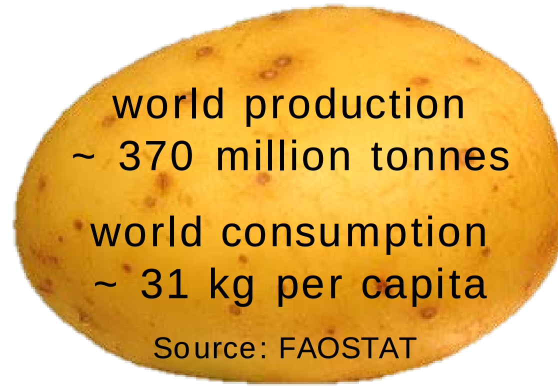
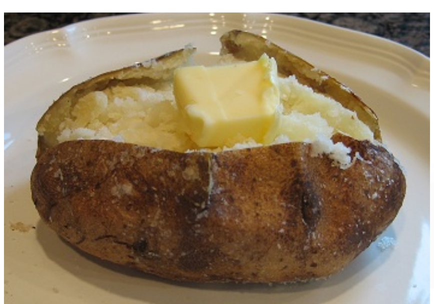

world production
~ 370 million tonnes
world consumption
~ 31 kg per capita
Source: FAOSTAT



COLORADO POTATO BEETLE

- Major potato pest worldwide

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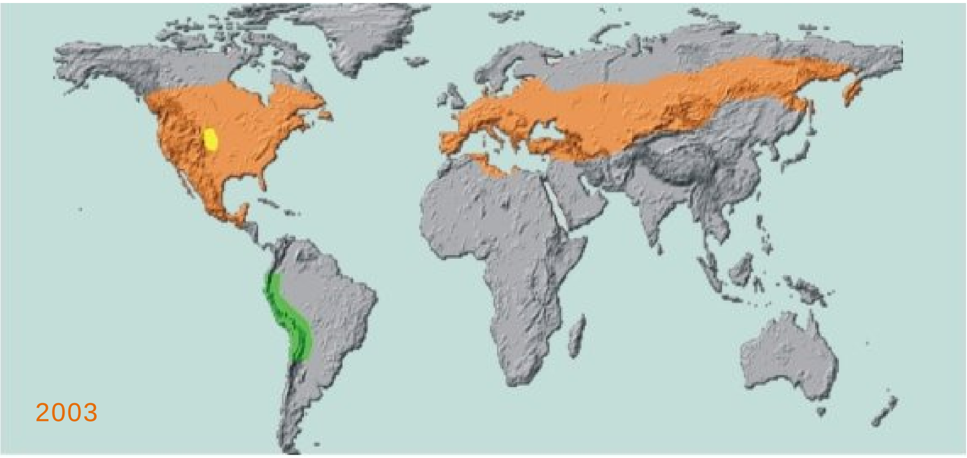

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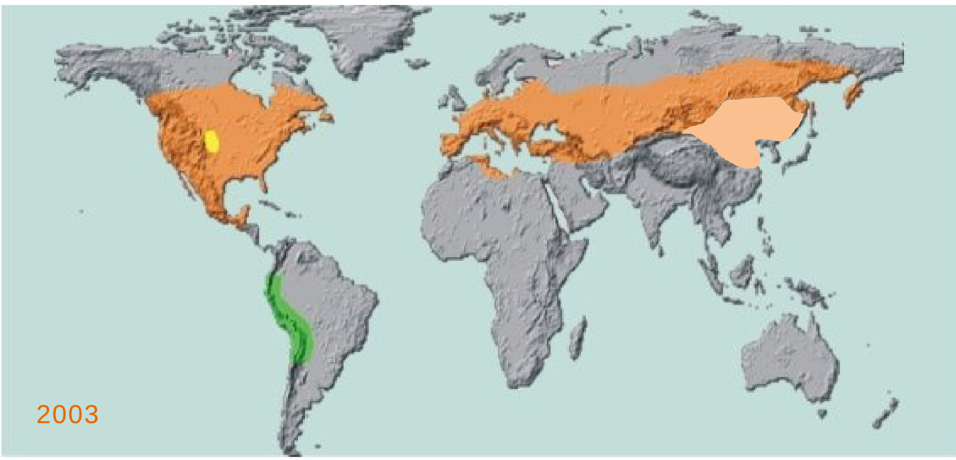

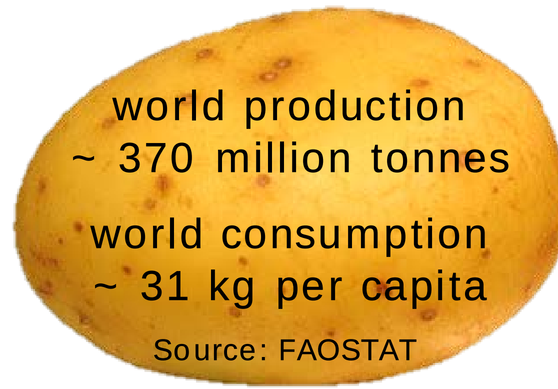

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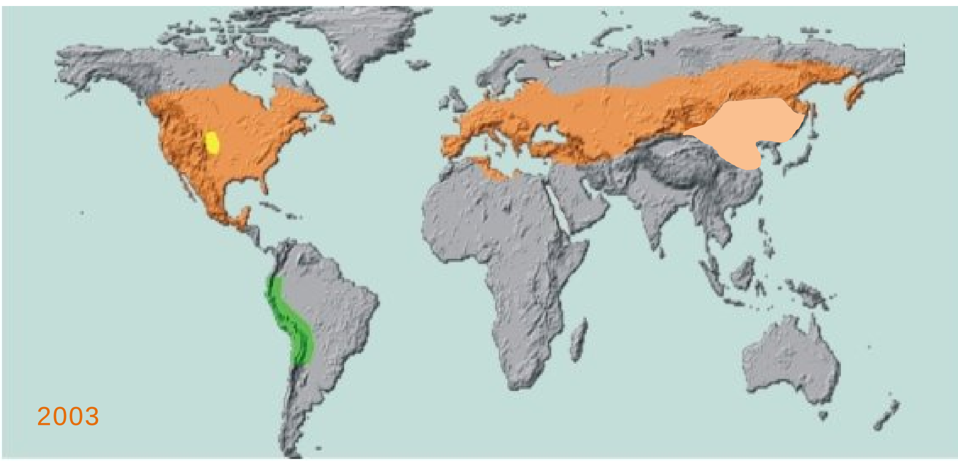
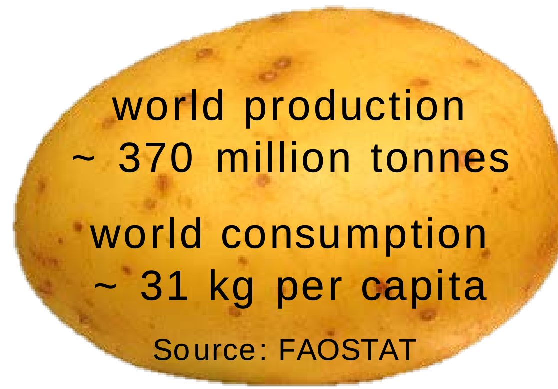
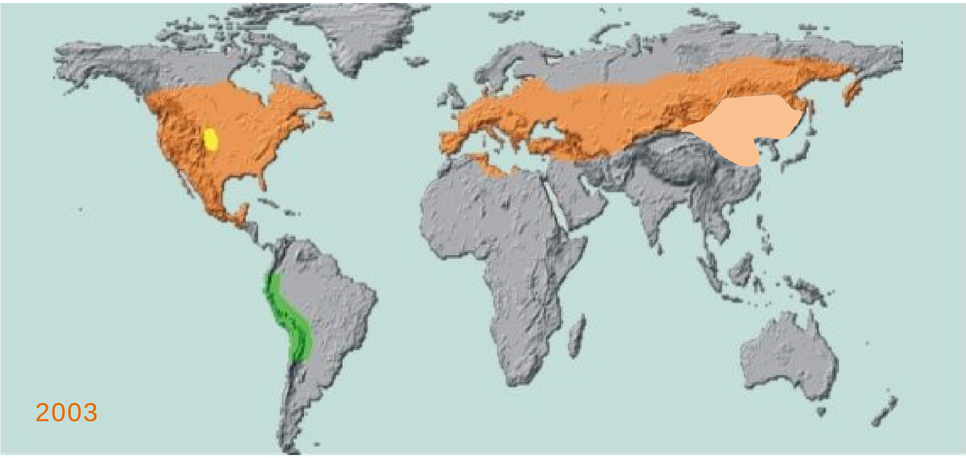


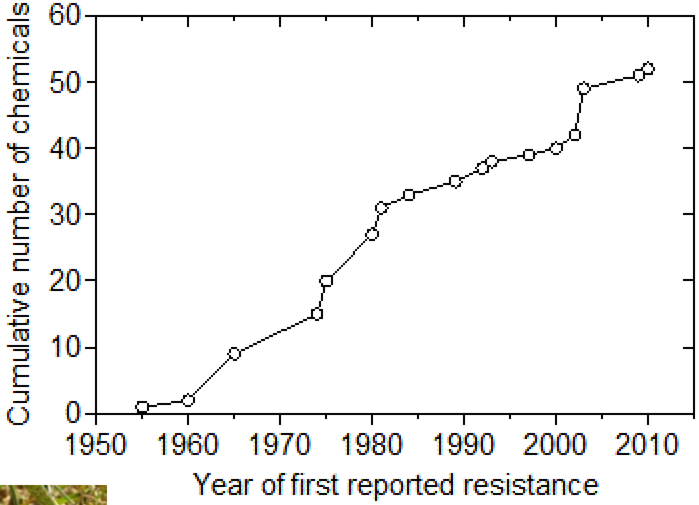


One larva consumes app. 40 cm^2 of potato leaves per day.
One adult beetle consumes app. 10 cm^2 of potato leaves per day.

COLORADO POTATO BEETLE

- Major potato pest worldwide
- Rapidly aquires resistance to pesticides





Source: Arthropod Pesticide Resistance Database, 2015
http://www.pesticideresistance.org/

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- Protease inhibitors of various sources considered for plant protection

MUSHROOMS
as a source of proteins with insecticidal properties

Mycocypins

cysteine protease inhibitors unique to basidiomycetes

Clitocypin and Macrocypins

Clitocybe nebularis

Clouded agaric

Macrolepiota procera

Parasol mushroom

Sabotič et al., Biological Chemistry 2006; Sabotič et al., Protein Expr Purif 2007; Sabotič et al., FEBS Journal 2009

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SIMILAR BIOCHEMICAL PROPERTIES

- small proteins (16.8 and 19.2 kDa)
- very stable proteins (temp, pH)

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- clitocypin: > 90% seq.id.
  - no influence on inhibitory activity or specificity
- macrocypins: five groups (Mcp1-5 with 75-86% seq.id.)
  - sequence varibility affects the inhibitory profile

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SIMILAR BROAD INHIBITORY PATTERN (C1, C13)

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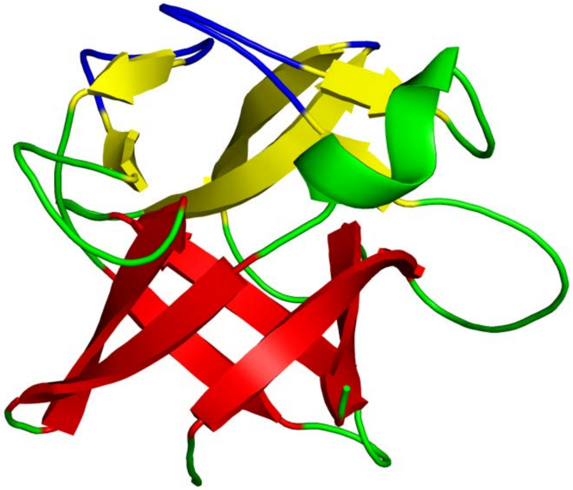

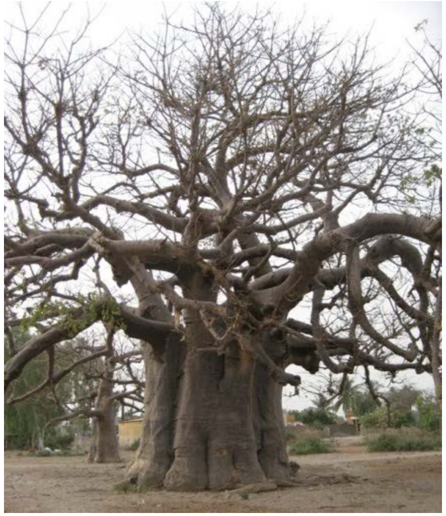

- same fold: β-trefoil fold
- clitocypin and macrocypins share ~ 20% seq. id.

Renko et al., JBC 2010; Sabotič et al., FEBS Journal 2009

Mycocypins

3D STRUCTURE AND MECHANISM OF INHIBITION

Renko and Sabotič et al.
JBC 2010


```
                      10        20        30        40        50        60        70        80        90
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cMcp1a        -MGFEDGFYTILHLAEGQHPNSKIPGGMYASSKDGKDVPVTAEPLGP--QSKIRWWIARDPQAGDDMYTITEFRIDNSIPGQWSRSPVET 87
gMcp2a        -MALEDGFYTLRHLAEDESPN--IPGGMYASSKDGKDVPVTAEPLGP--HGKIRWWIARHPEAGDDMYTITEFRVDKSIPGQWARSPIEV 85
cMcp3a        -MALEDGFYTLRHLAEGESPN--IPGGMYASSKDGKDVPVTAEPLGP--HSKIRWWIARDPAAGDDMYTITEIRDDNCIPGQWARSPRET 85
cMcp4a        -MALEDGFYTIRHLVEGQHPS--IPGGMYASSKDGKDEPVTAEPLGP--HSKIRWWIAAAPEAGDDMYTITEFRADKSIPGQWARSPTEI 85
gMcp5a        -MGFEDGFYTIRHLVEGQPPN--IPGGMYASSKDGKDEPVTAEPLGP--HSKIRWWIARYPEAGEDMYTITEFRVDESIPGQWARPHNEV 85
gClt-Kras     MASLEDGIYRLRAVTTHNPDP--GVGGEYAT-VEGARRPVKAEPNTPPFFEQQIWQVTRN---ADGQYTIKYQGLNTPFEYGFSYDELEP 84
cClt-Kras     MASLEDGTYRLRAVTTSNPDP--GVGGEYAT-VEGARQPVKAEPSTPPFFERQIWQVTRN---SDGQSTIKYQGLNAPFEYGFSYDQLEQ 84
cClt-Vrh      MASLEDGTYRLRAVTTHNPDP--GVGGEYAT-VEGARQPVKAEPNTPPFFERQYWQVTRN---ADGQYTIKYQGLNTPFEYGFSYDELEP 84
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                     100       110       120       130       140       150       160       170
              ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
cMcp1a        EVPVYLYDRIKAEETGYTCAWRIQPADHGADGVYHIVGNVRIGSTDWADLREEYGEPQVYMKPVPVIPN-VYIPRWFILGYEE 169
gMcp2a        GPPVYLYDRIKAEETGYTYFWRIQPTDEGADGVYHIMGNSRIGSTDWADLREEGGKPQVYTKPVPVIPN-VYIPRWFILGYEE 167
cMcp3a        EVPVYLYDRIKADEVGYVLVWKIQPAYEDVDGVYSIMGNVRIGSTDWADLREEDNKPQVYMKPVPVVPN-VYIPRWFISEVKR 167
cMcp4a        GVPVYLYDRIKAEETGYTCVWRIQPTYEGVGGVYNIMGNSRIGSTDWADLRGEDGKPQVYTKPVPVIPN-VYIPRWFISEYKE 167
gMcp5a        GGPVYLYDRIRAEETGYVCEWRIQPAYEDVDGVFNIMGNSRIGSTDWADLREEGGKPQVYLKPVPVVPN-MYIPRWFISKVD- 166
gClt-Kras     NAPVIAGD-------PKEYILQLVPSTADVYIIRAPIQ--RIG----VDVEVGVQGNTLVYKFFPVDGSGGDRPAWRFTRE-- 152
cClt-Kras     NAPVIAGD-------PKEYILQLVPSTTDVYIIRAPIQ--RVG----VDVEVGVQGNNLVYKFFPVDGSGGDRPAWRFTRE-- 152
cClt-Vrh      NAPVIAGD-------PKEYILQLVPSTTDVYIIRAPIQ--RVG----VDVEVGVQENTLVYKIFPVDGSGGDKAAWRFTRE-- 152
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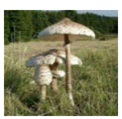
papain-like proteases



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                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
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gMcp2a          -MALEDGFYTLRHLAEDESPN--IPGGIYASSKDGKDVPVTAFPLGP--HGKIRWWIARHPEAGDDMYTITEFRVDKSIPGQWARSPIEV 85
cMcp3a          -MALEDGFYTLRHLAEGESPN--IPGGIYASSKDGKDVPVTAFPLGP--HSKIRWWIARDPAAGDDMYTITEIRDDNCIPGQWARSPRET 85
cMcp4a          -MALEDGFYTIRHLVEGQHPS--IPGGIYASSKDGKDEPVTAFPLGP--HSKIRWWIAAAPEAGDDMYTITEFRADKSIPGQWARSPTEI 85
gMcp5a          -MGFEDGFYTIRHLVEGQPPN--IPGGIYASSKDGKDEPVTAFPLGP--HSKIRWWIARYPEAGEDMYTITEFRVDESIPGQWARPHNEV 85
gClt-Kras       MASLEDGIYRLRAVTTHNPDP--GVGGLYAT-VEGARRPVKAFPNTPPFFEQQIWQVTRN---ADGQYTIKYQGLNTPFEYGFSYDELEP 84
cClt-Kras       MASLEDGTYRLRAVTTSNPDP--GVGGLYAT-VEGARQPVKAFPSTPPFFERQIWQVTRN---SDGQSTIKYQGLNAPFEYGFSYDQLEQ 84
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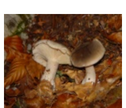

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cMcp4a          GVPVYLYDRIKAEETGYTCVWRIQPTYEGVGGVYNIMGNSRIGSTDWADLRGEDGKPQVYTKPVPVIPN-VYIPRWFISEYKE 167
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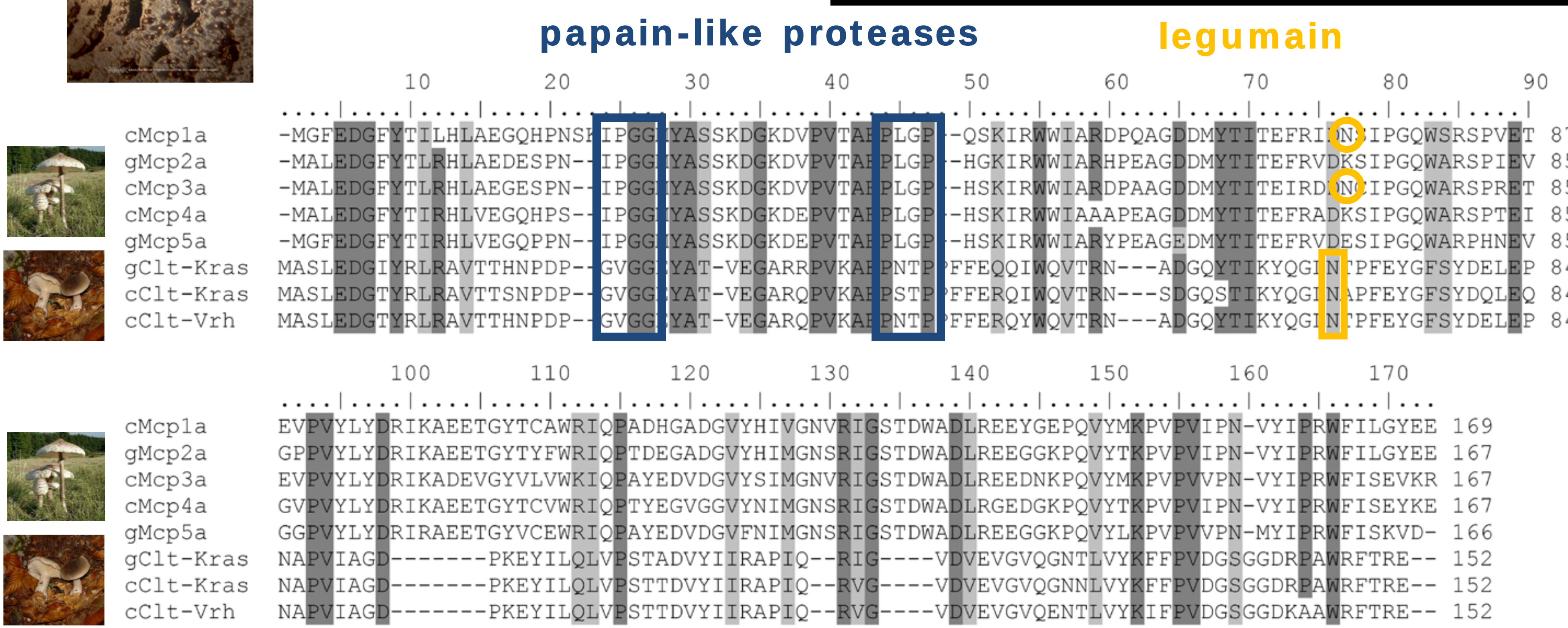

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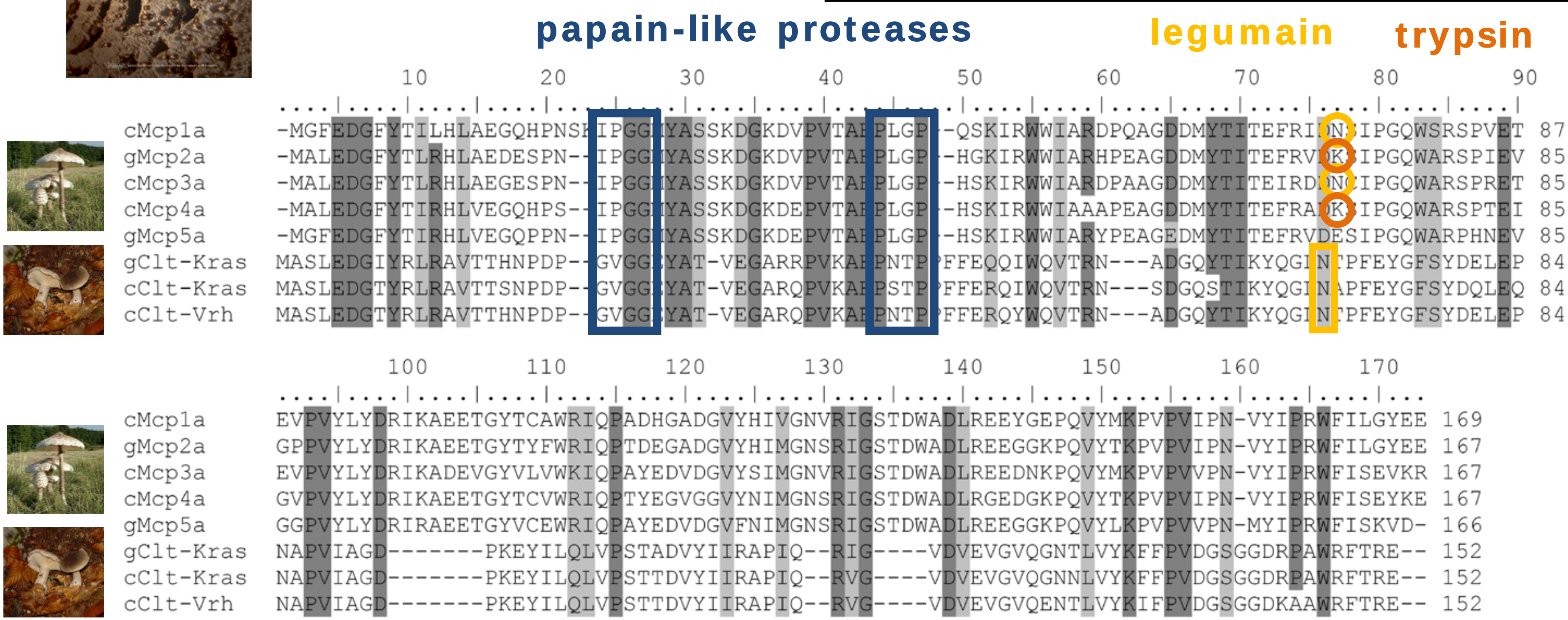

Mycocypins

- Small proteins resistant to extreme conditions (pH & temp)
- Resistant to proteolytic degradation
- Inhibitors of cysteine proteases (broad spectrum)

Šmid et al., J Agric Food Chem 2013; Šmid et al., Pestic Biochem Physiol 2015

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> Analysis of gene expression of known adaptation-related digestive enzymes in larval guts

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Macrocypins vs. Colorado potato beetle

- Transgenic potato expressing macrocypin 4 used in feeding assays

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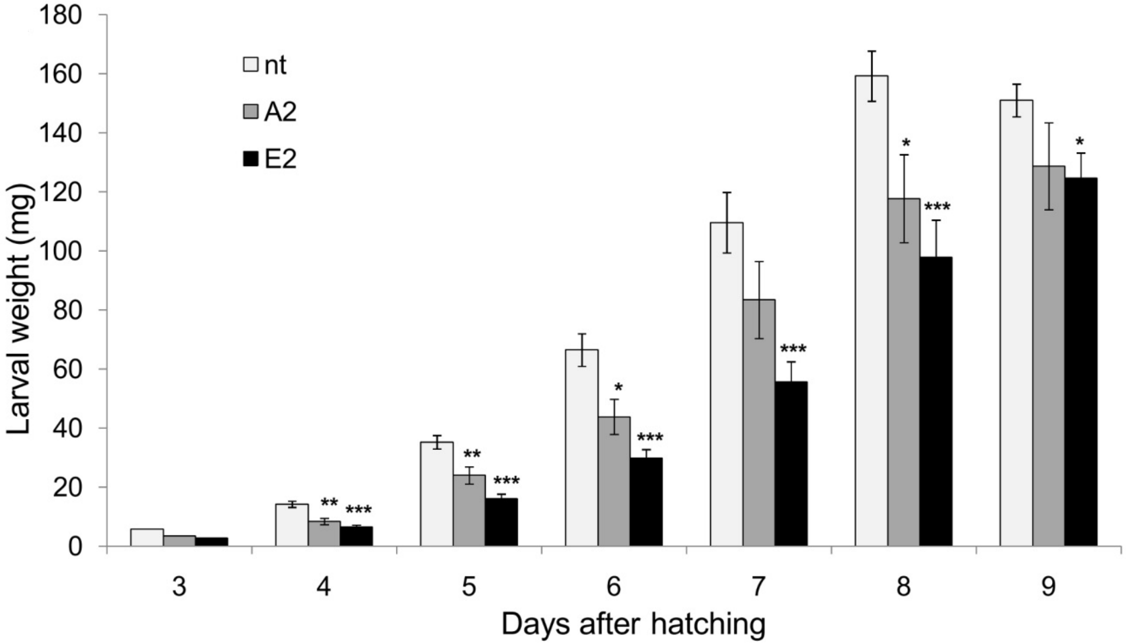

Macrocypin 4 negatively affects weight gain of CPB larvae.

Šmid et al., J Agric Food Chem 2013

Macrocypins vs. Colorado potato beetle

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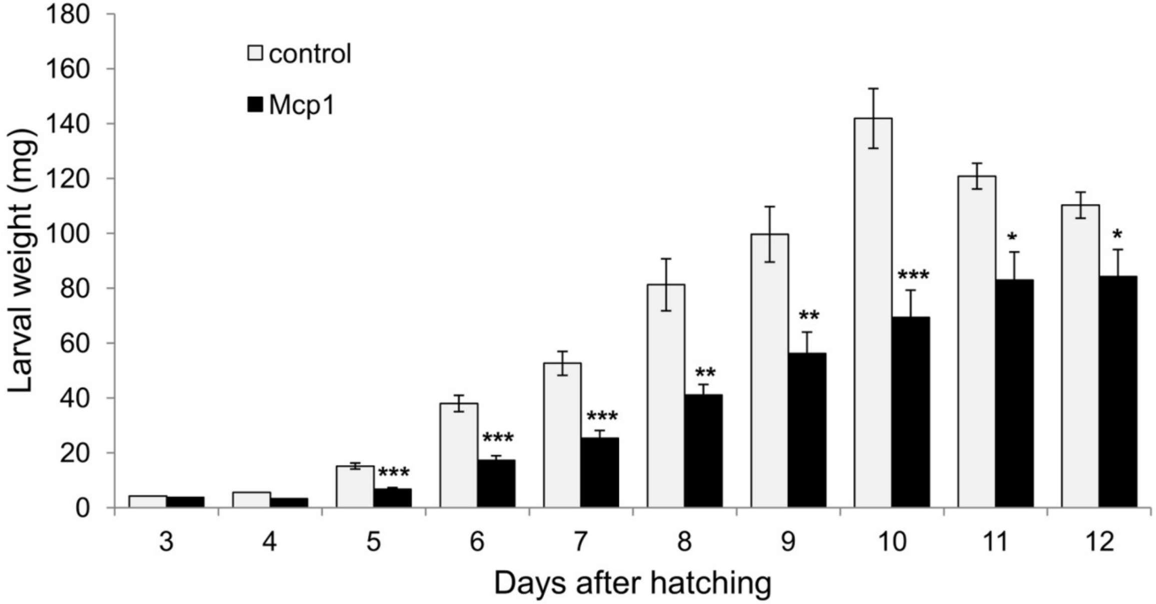

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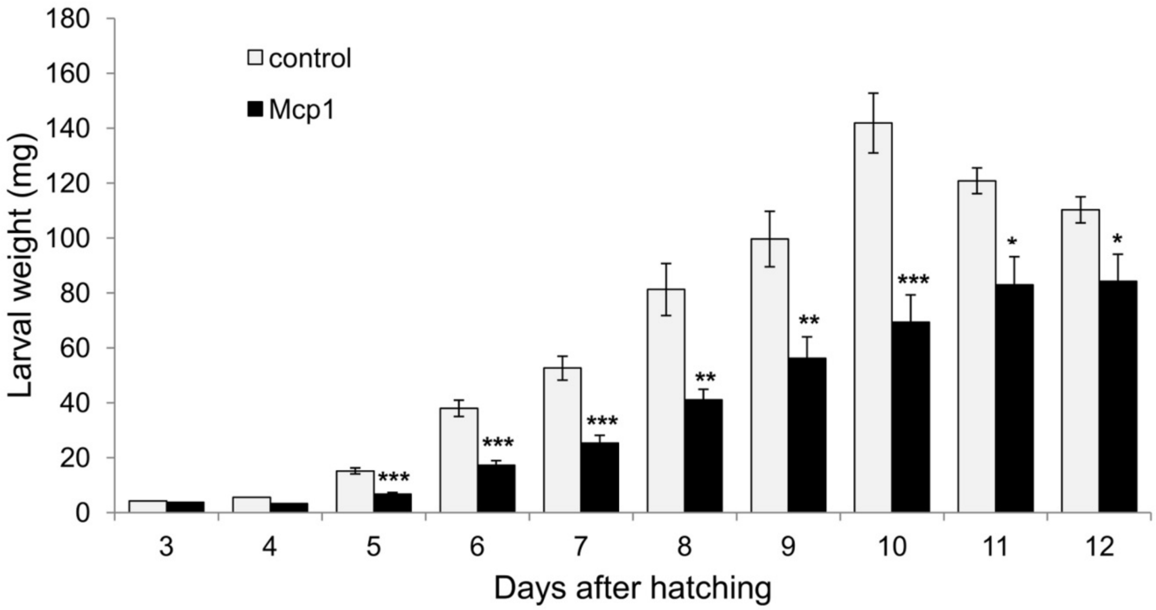


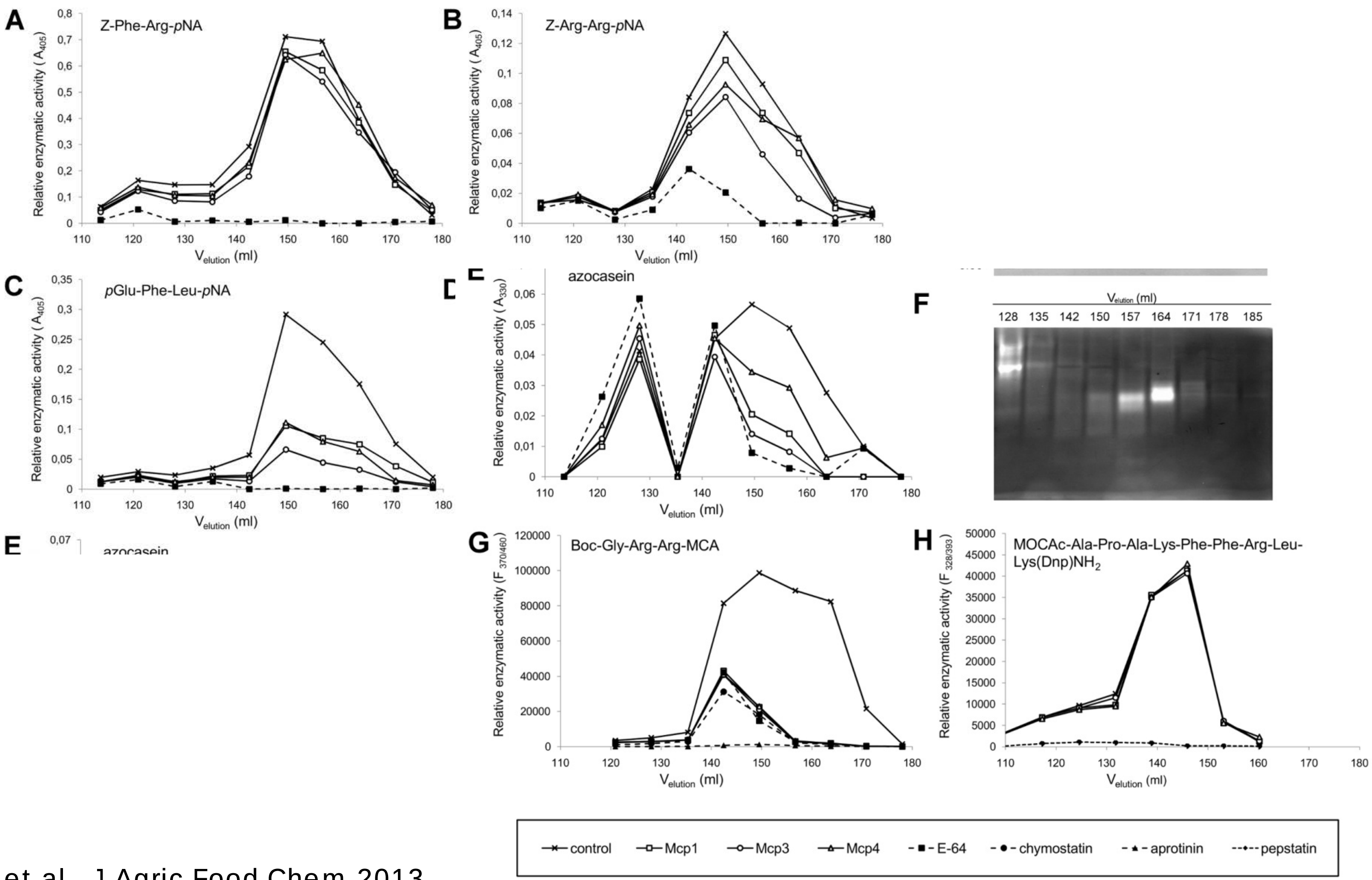
Macrocypin 1 significantly lowers weight gain of CPB larvae.

What is the mechanism for the negative effect?

Šmid et al., J Agric Food Chem 2013

Macrocypins vs. Colorado potato beetle

Enzymatic tests using fractionated protein extract from CPB larval guts



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- Macrocypins inhibit a specific subset of digestive cysteine proteases intestains

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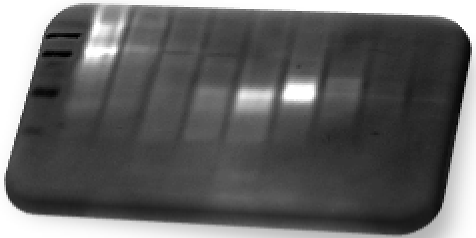

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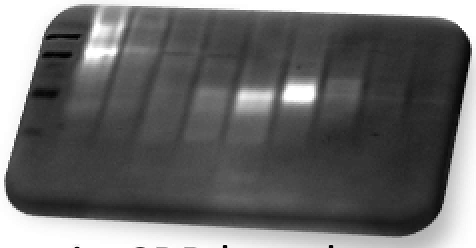

International patent application: Istinič, Sabotič, Gruden, Brzin, Buh Gašparič, Žel. »Use of macrocypins as pesticidal agents« (PCT/EP2012/065373)

Šmid et al., J Agric Food Chem 2013

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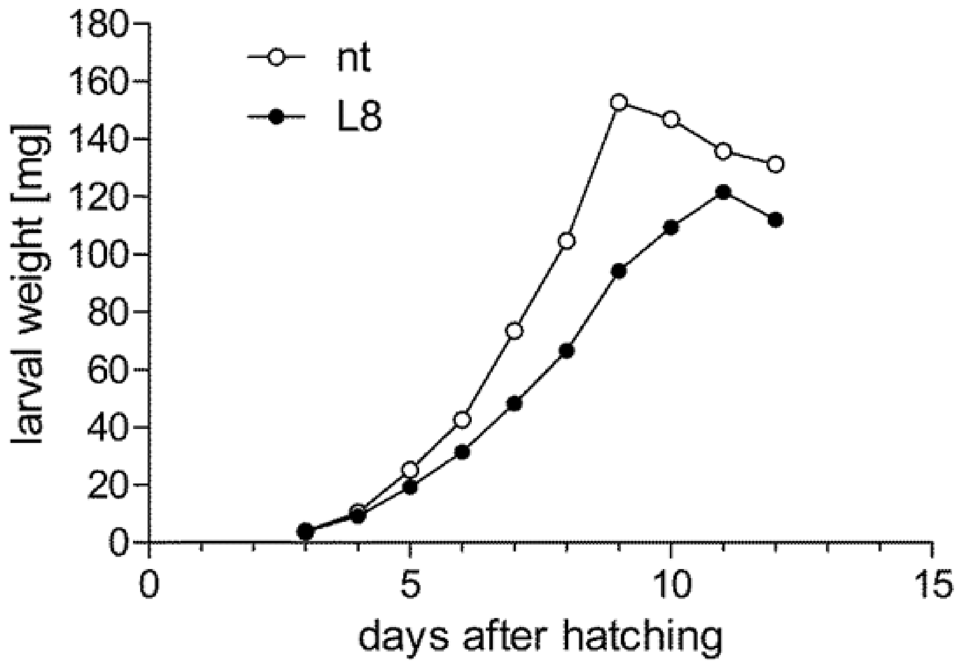

Clitocypin negatively affects weight gain of CPB larvae.

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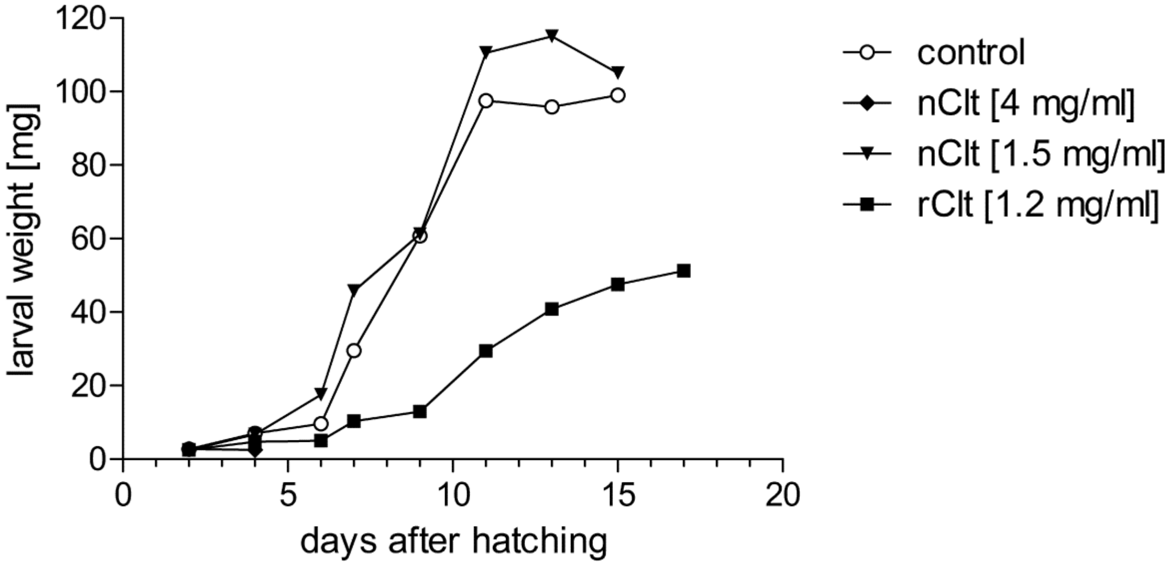

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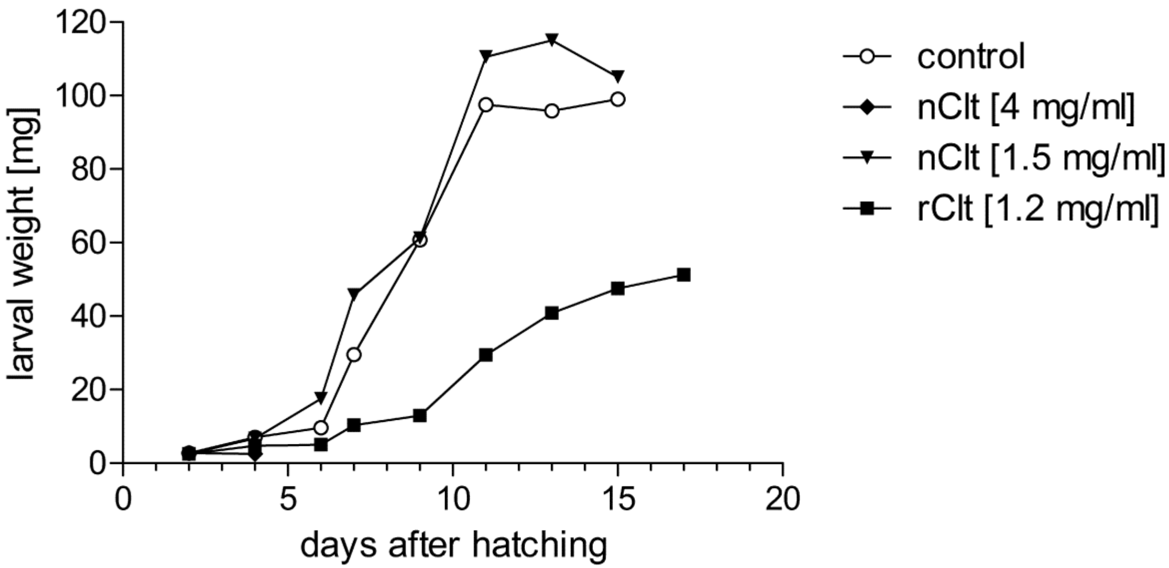

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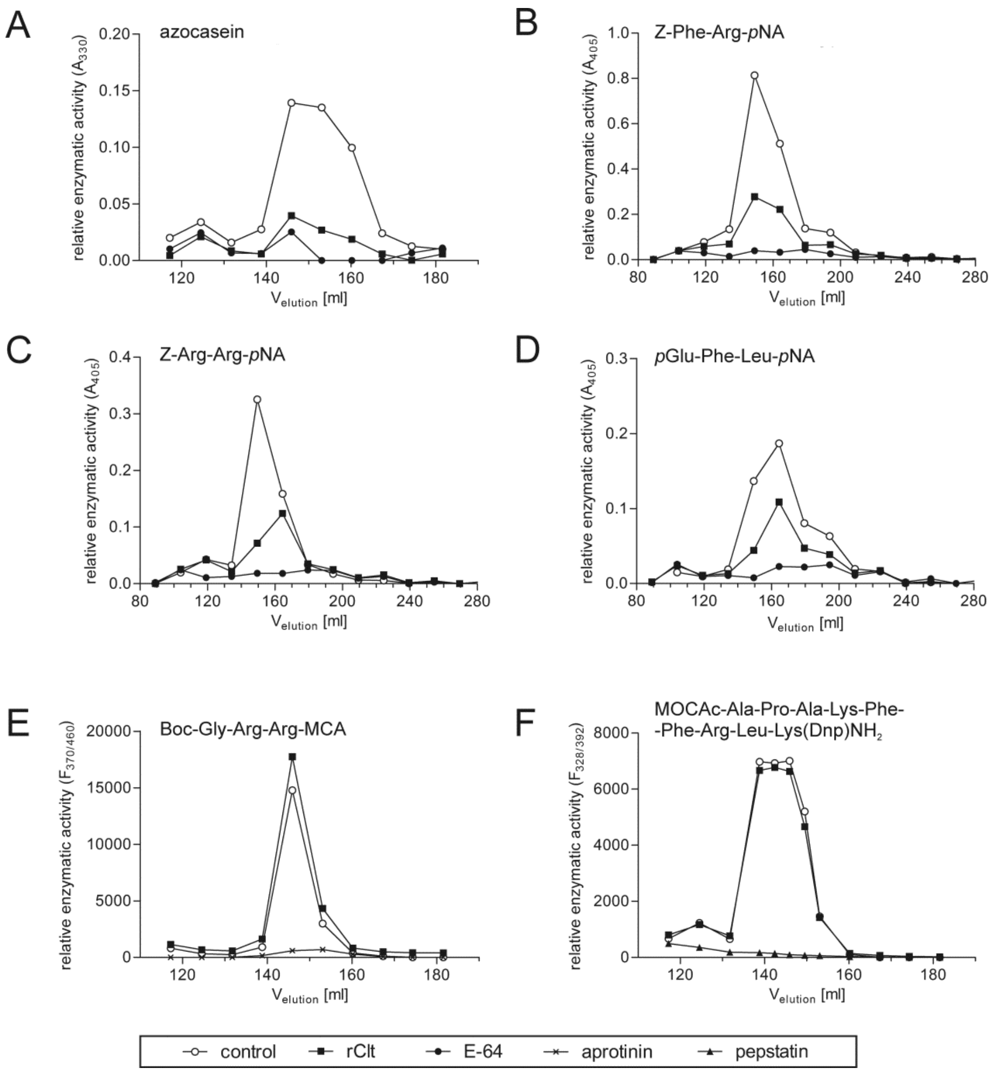

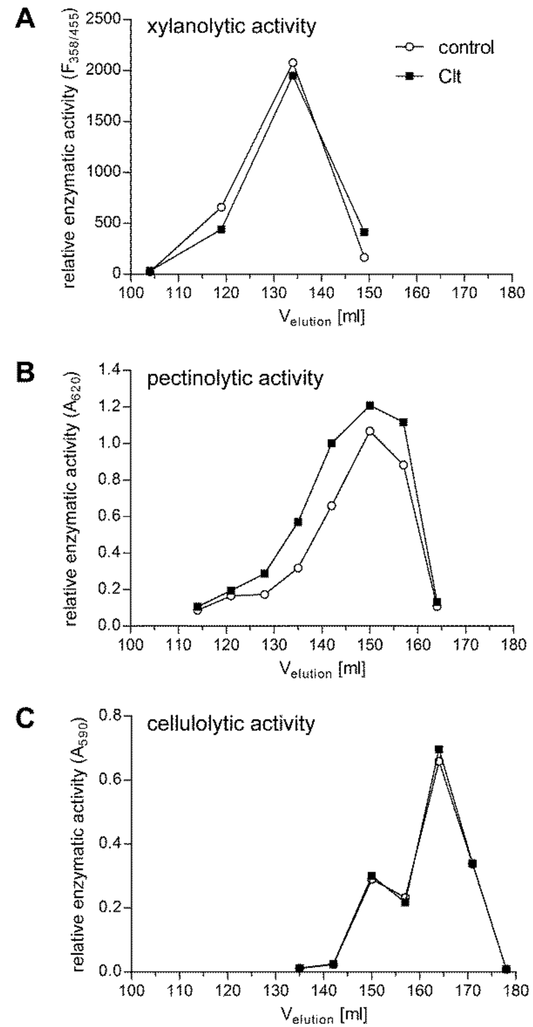

Šmid et al., Pestic Biochem Physiol 2015

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- Clitocypin does not elicit an expected adaptation response in CPB larval gut at transcriptional level.

Šmid et al., Pestic Biochem Physiol 2015

MUSHROOMS are a rich source of potential insecticidal / phytoprotective proteins

ADVANTAGES:

- Different
- Unique molecular features
    - Stable protein scaffold
    - Specificity and selectivity
    - Resistance to proteolytic digestion
- Diversity offering different mechanisms of insecticidal activity
- Evolutionary distance

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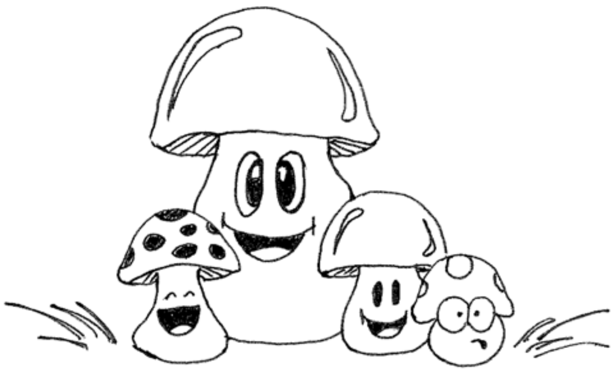

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