

insect pests, the addition of selective inhibitors could provide a useful method to avoid capture of non-target insects that can cause unnecessary alarm to growers.

From the knowledge of inhibitors, no valid conclusions can at present be drawn on structures of sex pheromones. It is nevertheless interesting that 3 *H. nubiferana* inhibitors found in this study, *cis*-7 through *cis*-9-dodecenyl acetate, are also reported to be inhibitors of sex attraction in *Rhyacionia buoliana*<sup>10</sup>.

Some compounds seemed to increase catches of *L. pomonella* (9 and 27) or of *H. nubiferana* (10 and 12) over the control, but this effect was in no case significant.

A number of other lepidoptera were specifically attracted to traps containing certain chemicals; whether the presence of Codlemone affected their catch is unknown. *Celypha striana* was attracted to *cis*-8-dodecen-1-ol (15), confirming an earlier observation<sup>11</sup>. Attraction of a *Bryotropha* species to *trans*-9-tetradecenyl acetate (29) has been observed in North America<sup>12</sup>. Of special interest is the capture of *Spilonota ocellana*, a widespread apple-feeder. About 100 males of this species were caught between June 27 and July 11 in 4 traps containing *cis*-8-tetradecenyl acetate (26). Catches of this species and those of *Apotomis corticana* and *Endothenia carbonana* with another 14-carbon compound (30) indicate that the attraction of *Olethreutinae* to 12-carbon compounds<sup>13</sup> may not be a general rule. Several *G. funebrana* males were

caught in traps containing *L. pomonella* females and *cis*-10-dodecenyl acetate. Although no direct comparison could be made, this compound seemed less attractive than *cis*-8-dodecenyl acetate.

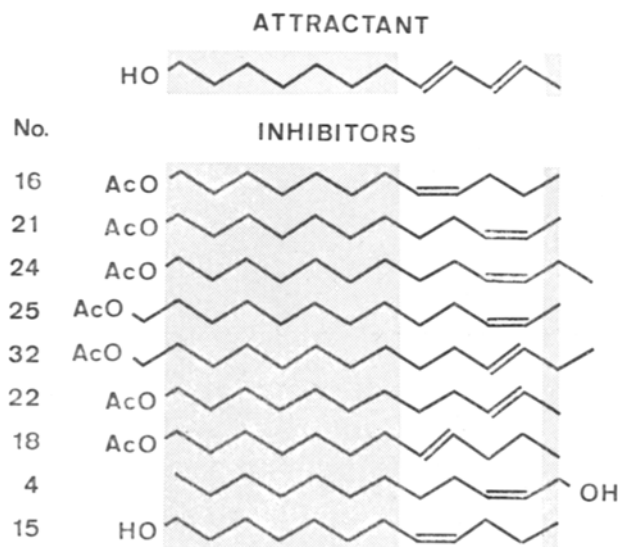
Conclusive evidence for the use of inhibitors in insect control has not yet been established. In a preliminary test in connection with a plum fruit moth confusion experiment<sup>14</sup>, evaporation of *cis*-8-dodecenyl acetate from several sources within an apple orchard at the rate of ca. 150 to 600 mg per day and hectare did not prevent codling moth males from being recaptured in Codlemone- or female-baited traps. Similar results were obtained in Australia with the oriental fruit moth and the inhibitor dodecyl acetate<sup>15</sup>. On the other hand, orientation of codling moth males to females was successfully prevented over a short period with relatively large doses of *trans*-8, *trans*-10-dodecadienyl acetate<sup>8</sup>.

Many insights into the mechanism of sex attractant inhibition were obtained with compounds that recently proved to be synergistic at low levels<sup>16</sup>. Further studies will be needed to determine the actual effect of inhibitors on the various phases of mating behavior.

**Zusammenfassung.** Die Anlockung von Männchen des Apfelwicklers (*Laspeyresia pomonella* L.) mit *trans*-8, *trans*-10-Dodecadien-1-ol (Codlemone) oder mit lebenden Weibchen wird durch verschiedene Analoge des Sexuallockstoffs gehemmt. Starke Inhibitorwirkung zeigte *cis*-8-Dodecenylacetat. Der graue Knospenwickler, *Hedya nubiferana* Haw., der ebenfalls durch Codlemone angelockt wird, reagiert spezifisch auf weitere Inhibitoren.

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Structural similarities between codling moth sex attractant and inhibitors.

<sup>10</sup> R. LANGE and D. HOFFMANN, *Naturwissenschaften* 59, 217 (1972). – G. E. DATERMAN, G. D. DAVES, M. JACOBSON, *Envir. Entom.* 1, 382 (1972).

<sup>11</sup> J. GRANGES and M. BAGGIOLINI, personal communication.

<sup>12</sup> W. L. ROELOFS and A. COMEAU, *Science* 165, 398 (1969).

<sup>13</sup> A. COMEAU and W. L. ROELOFS, *Entomologia exp. appl.* 16, 191 (1973).

<sup>14</sup> H. ARN, B. DELLEY, M. BAGGIOLINI and P. CHARMILLOT, in preparation.

<sup>15</sup> G. H. L. ROTHSCHILD, *Entomologia exp. appl.* 17, 294 (1974).

<sup>16</sup> J. A. KLUN, O. L. CHAPMAN, K. C. MATTES, P. W. WOJTKOWSKI, M. BEROZA, P. E. SONNET, *Science* 181, 661 (1973). – M. BEROZA, G. M. MUSCHIK and C. R. GENTRY, *Nature New Biol.* 244, 149 (1973).

### Some Observations on the Seed Coat Structure within the Genus *Epilobium*

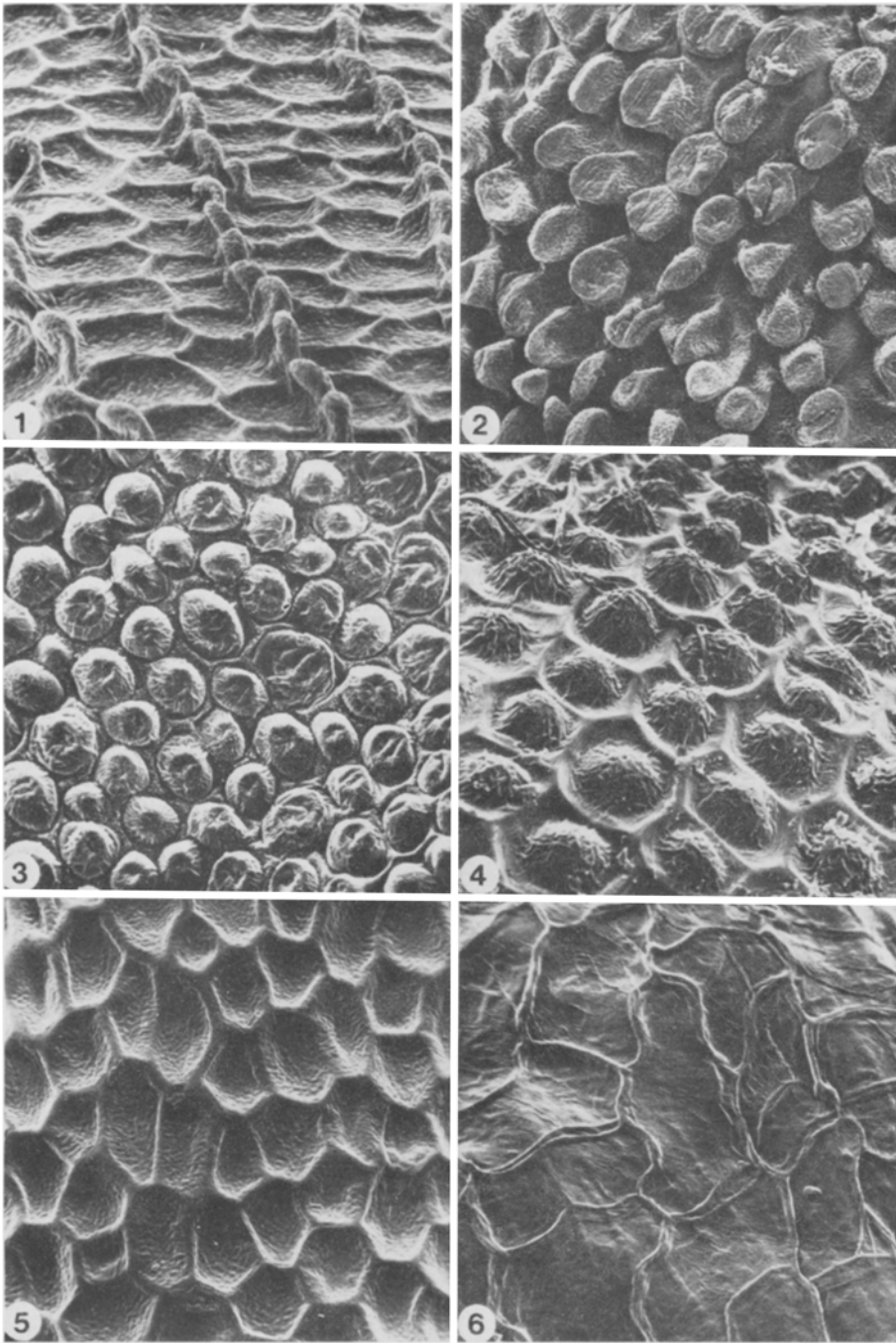
The genus *Epilobium* is a very variable one whose species exhibit a wide range of morphological flexibility<sup>1</sup>. It is not surprising then to read in the literature that there may well be over 200 species throughout the world within this genus<sup>1-3</sup>. Furthermore the complexity of the genus has led to much difficulty in determining the distribution patterns of the various taxa, simply because of the lack of knowledge of the total variability within species<sup>3</sup>.

Despite the variable nature of the genus, autopolyploidy, rather than allopolyploidy, seems to occur in such variable species as *E. latifolium* and *E. angusti-*

<sup>1</sup> P. A. MUNZ, in *North American Flora II* (W. H. Scripps, Claremont, California, USA 1965), part 5, p. 1-231.

<sup>2</sup> P. H. RAVEN, *Bull. Br. Mus. Nat. Hist. Bot.* 2, 325 (1962).

<sup>3</sup> E. HULTÉN, *Flora of Alaska and Neighbouring Territories* (Stanford University Press, California 1968).



Figs. 1-6. S.E.M. seed coat ornamentation.  $\times 900$ .

*folium*<sup>4,5</sup>. Cytotaxonomically the general chromosome number is  $n = 18$ , exceptions in North America do occur with such counts as  $n = 11, 12, 13$  and  $15$ <sup>6</sup>. Such variability in chromosome number (and inevitably ploidy) seems to be restricted to North Western America, specifically Nevada and the northern coastal regions of California.

Distributionally the genus is quite cosmopolitan and many species are circumboreal. Ecologically there seems to be little to indicate habitat specificity (many are weeds) with the possible exception of *E. latifolium* which appears to grow in abundance on gravel bars, although it is also found in sup-alpine regions, and *E. hornemanii* which often is found in flowing waterlogged areas.

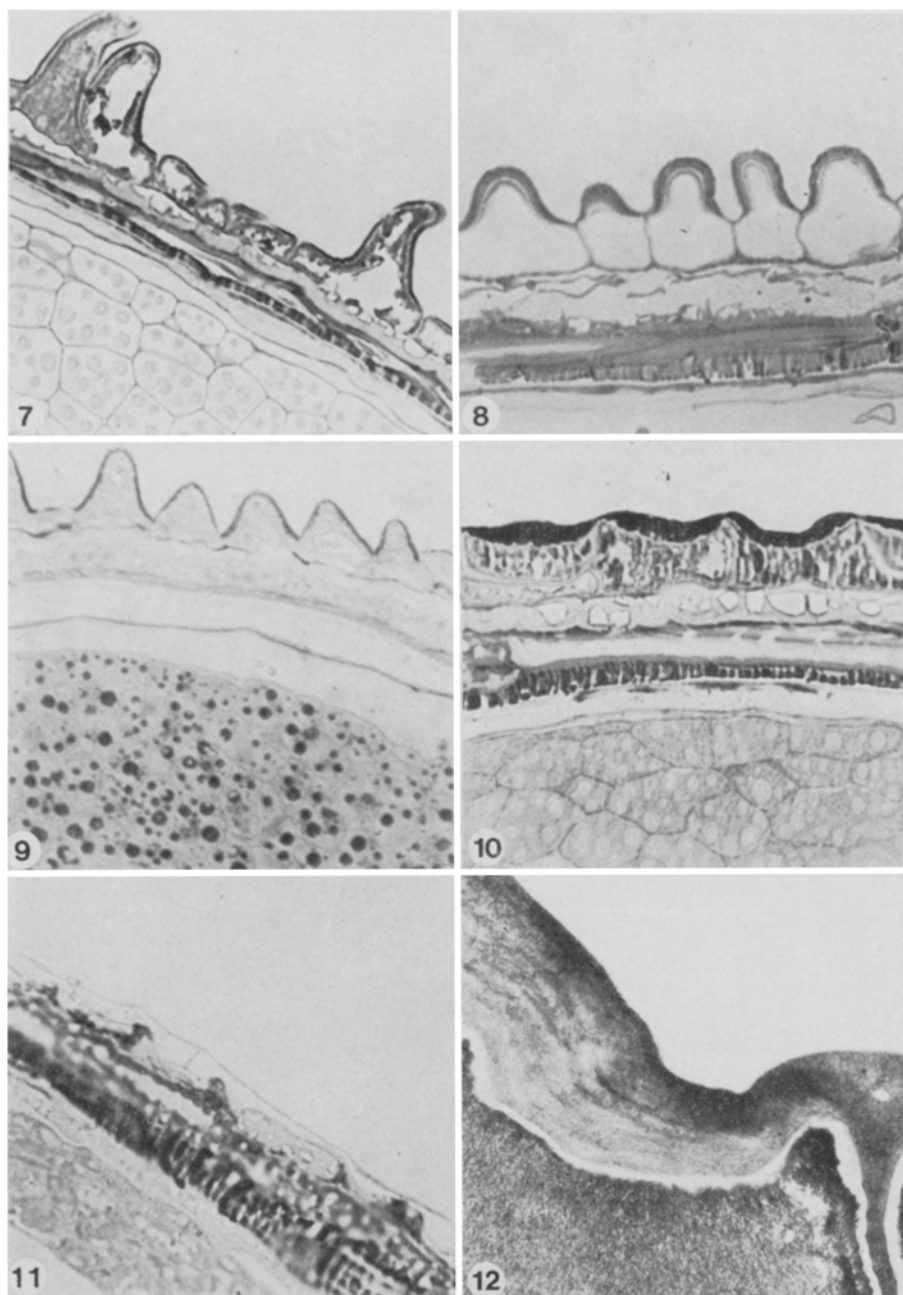
One area of investigation which seems to have been overlooked is the constancy of the seed coat characteristics. Several workers have suggested that seed coat structure may well be of value in rather otherwise variable groups, because of the constancy of this character<sup>7</sup>. Indeed within the genus *Epilobium* seed coat

<sup>4</sup> T. MOSQUIN, *Brittonia* 18, 167 (1966).

<sup>5</sup> E. SMALL, *Brittonia* 20, 169 (1968).

<sup>6</sup> H. LEWIS and P. H. RAVEN, *Records Advanced Botany* (University of Toronto Press, Toronto 1961), vol. 2, p. 1466.

<sup>7</sup> P. H. DAVIS and V. H. HEYWOOD, *Principles of Angiosperm Taxonomy* (Olivier and Boyd, Edinburgh and London 1963).



Figs. 7-11. Light microscope sections.  $\times 1,125$ .

Fig. 12. E.M. section papilla wall.  $\times 24,375$ .

sculpturing is used for certain species identification (papillate vs. smooth).

In this study, the seed coat structure of 17 North American taxa within the genus have been investigated using light stereo-microscopy, scanning electron microscopy and seed coat sectioning, to ascertain more critically the value of seed coat structure within the genus.

The seeds of the taxa under investigation were obtained both from the field and the University of Alberta Herbarium. They were fixed for 12 h at  $0^{\circ}\text{C}$  in 3% glutaraldehyde in 0.025 M phosphate buffer pH 6.9. After several buffer series the seeds were post fixed for 3 h in 2% buffered osmium tetroxide at  $0^{\circ}\text{C}$ . Dehydration was carried out with a graded series of ethanol. The tissues were embedded in SPURR's resin<sup>8</sup>.

Thick sections, 1-1.5  $\mu\text{m}$ , were cut with a glass knife on a Reichert OMU<sub>2</sub> ultra-microtome and collected on

gelatin coated glass slides<sup>9</sup>. Periodic acid-Schiff's<sup>10</sup> and aniline blue black<sup>11</sup> procedures were used in order to demonstrate the morphology of the seed coat. Photographs were obtained with a Zeiss photomicroscope using Kodak Plus X Pan film.

Electron micrographs of the cell wall structure (Figure 12) was obtained from ultra thin sections cut from the same blocks used for light microscopy. The sections were stained sequentially with aqueous uranyl acetate and

<sup>8</sup> A. R. SPURR, J. Ultrastruct. Res. 26, 31 (1969).

<sup>9</sup> W. A. JENSEN, *Botanical Histochemistry* (W. H. FREEMAN & Co, San Francisco 1962).

<sup>10</sup> R. D. HOTCHKISS, Arch. Biochem. 16, 131 (1948).

<sup>11</sup> D. B. FISHER, Histochemie 16, 92 (1968).

lead citrate<sup>12</sup>, and viewed under a Phillips 200 E.M. Seeds from the same source were glued to E.M. stubs and coated sequentially with 100 Å thick layer of carbon and gold and viewed with a Cambridge stereoscan S4.

Under both the light microscope and S.E.M., it was very apparent that the taxa investigated fall into 2 groups with respect to their seed coat structure, papillate and smooth. However, within both of these categories it is apparent also that papillation is of 2 types, as is the nature of the so called smooth seeded group.

The 2 types of papillae present are illustrated in Figures 2 and 4. In one the whole of the epidermal cell appears to be modified into a papilla and is substantially thickened on its outer wall. This is noticeable under light microscope sections Figure 8 and also the layered structure is clearly visible under transmission E.M. (Figure 12). The other type of papillation, for convenience referred to as sub-papillate, involves only part of the cell wall (Figure 4). The central region of the outermost part of the epidermal cell is modified into a papilla, but the surface also appears to be foveolate (Figures 3 and 4).

#### Keys to Figures 1–12

Seed coat structure	Species	Figures
Papillate	<i>E. glandulosum</i>	1, 7
	<i>E. platyphyllum</i> Rydb.	2, 8, 12
	<i>E. hornemannii</i> Reichenb.	2, 8, 12
	<i>E. clavatum</i> Trel.	2, 8, 12
Sub-papillate	<i>E. paniculatum</i> Nutt.	3, 9
	<i>E. hirsutum</i>	3, 9
	<i>E. anagallidifolium</i> Lam.	3, 9
	<i>E. davuricum</i> Fisch.	4, 8
	<i>E. leptophyllum</i> Raf.	4, 8
	<i>E. palustre</i> L.	4, 8
	<i>E. palustre</i> var. <i>grammadophyllum</i>	4, 8
Smooth	<i>E. angustifolium</i> L.	6, 11
	<i>E. latifolium</i> L.	6, 11
Foveolate	<i>E. lactiflorum</i> Hausskn.	5, 10
	<i>E. alpinum</i> var. <i>alpinum</i>	5, 10
	<i>E. luteum</i> Pursh	5, 10

In the case of *E. glandulosum*, the papillae are arranged in rows and have 2 almost foveolate cells between them making this taxon particularly distinctive. At the same time, however, the papilla is a complete cell, not just a modified outer wall (Figures 1 and 7).

The smooth or non-papillate seed coat type is of 2 forms (Table, Figures 5 and 6). One as is exemplified by *E. latifolium* and *E. angustifolium* is only superficially sculptured (Figure 6) whereas the other is foveolate, the centre of the cell being sunken and somewhat alveolate in its sculpturing. In sections (Figures 7–11) these seed surfaces, especially the epidermal cells, are very different. The *latifolium-angustifolium* type has unthickened outer wall (Figure 11), whereas the foveolate types have extremely thickened outer wall, almost to the extent that the epidermal cell contents are excluded (Figure 10).

The distinct differences between these seed coat types indicates a possible genetic discontinuity both within and between species. If the genetic relationship between different seed coat morphologies is a simple one, and there is every reason to think so because of the lack of 'intermediate' forms, then it should be possible to demonstrate biochemical affinities between taxa. Such investigations are underway based on the above findings with respect to the flavonoid and isoenzyme profiles of the taxa investigated.

**Résumé.** Nous avons examiné au MEB et au microscope ordinaire l'épiderme de la graine de 17 formes du genre *Epilobium* provenant de l'Amérique du Nord et relevé l'utilité et la valeur taxonomique et génétique des données obtenues dans le cadre du genre.

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16 April 1974.

<sup>12</sup> E. S. REYNOLDS, J. Cell Biol. 17, 208 (1963).

<sup>13</sup> Acknowledgments: This work was supported in part by the NRC of Canada and the Boreal Institute of Alberta.

### Inhibition of *Neisseria catarrhalis* NE-11 Transformation

The incorporation of antibiotics or other chemical inhibitors into transformational mixtures has been increasingly reported in recent years. These studies have demonstrated the importance of the involvement of cellular processes as energy utilization and protein, DNA, RNA, or cell wall synthesis in the process of transformation.

That DNA uptake is mediated by an energy-requiring process was first demonstrated by STUY<sup>1</sup> when he showed that *Hemophilus influenzae* transformation could be inhibited with either 2,4-dinitrophenol or sodium arsenate. STRAUSS<sup>2</sup> found that cyanide, an inhibitor of aerobic energy-yielding reactions and of membrane transport requiring energy, could block transformation of *Bacillus subtilis* apparently by preventing the transport of P<sup>32</sup> DNA into the competent cell. This led him to believe that transforming DNA in its DNase-insensitive stage was not necessarily inside the cell but may be located in an extramembranal space beneath the cell wall.

LIE<sup>3</sup> and JYSSUM<sup>4</sup> agreed that an energy source, as well as a certain level of metabolic activity, was required for the transformation of *Neisseria meningitidis*. JYSSUM<sup>4</sup> showed this by the finding that dinitrophenol inhibited *N. meningitidis* transformation.

Chloramphenicol, an antibiotic which inhibits the transfer of amino acids from aminoacyl transfer RNA to a growing polypeptide chain<sup>5</sup>, has been shown by many investigators to inhibit the development of competence. This resulted in a reduced frequency transformation in

<sup>1</sup> J. H. STUY, J. gen. Microbiol. 29, 537 (1962).

<sup>2</sup> N. STRAUSS, J. Bact. 101, 35 (1970).

<sup>3</sup> S. LIE, Acta path. microbiol. scand. 64, 119 (1965).

<sup>4</sup> K. JYSSUM, Acta path. microbiol. scand. 77, 477 (1969).

<sup>5</sup> B. D. DAVIS, R. DULBECCO, H. N. EISEN, H. S. GINSBERG and W. B. WOOD, Microbiology (Harper and Row, New York 1973).